Summer 7-5-2014

Evolutionary Divergence in Sexual Signals: Insights from within and among Barn Swallow Populations

Matthew Reed Wilkins
University of Colorado Boulder, m.r.wilkins06@gmail.com

EVOLUTIONARY DIVERGENCE IN SEXUAL SIGNALS: INSIGHTS FROM WITHIN AND AMONG BARN SWALLOW POPULATIONS

by

MATTHEW REED WILKINS
B.A., Vanderbilt University, 2006

A thesis submitted to the
Faculty of the Graduate School of the
University of Colorado in partial fulfillment
of the requirement for the degree of
Doctor of Philosophy
Department of Ecology and Evolutionary Biology
2014

This thesis entitled:
Evolutionary divergence in sexual signals:
Insights from within and among barn swallow populations
written by Matthew Reed Wilkins
has been approved for the Department of Ecology and Evolutionary Biology

_____
(Dr. Rebecca J. Safran, Committee Chair)

_____
(Dr. Michael Breed)

_____
(Dr. Deane Bowers)

Date_____

The final copy of this thesis has been examined by the signatories, and we find that both the content and the form meet acceptable presentation standards of scholarly work in the above mentioned discipline.

Matthew Reed Wilkins (Ph.D., Ecology and Evolutionary Biology)

Evolutionary divergence in sexual signals: Insights from within and among barn swallow populations

Thesis directed by Associate Professor Rebecca J. Safran

ABSTRACT

A wealth of studies across diverse animal groups indicate the importance of sexual selection in shaping phenotypes within and across breeding populations. In recent decades, much research has focused on how divergent sexual selection pressures among populations may lead to speciation. For my first dissertation chapter, I performed a literature review on the causes and consequences of evolutionary divergence in acoustic signals and developed the acoustic window conceptual framework for understanding the contributions of selection, genetic drift, and evolutionary constraint to signal divergence. Further, I found that sexual selection explains acoustic differences between recently diverged populations of the best-studied taxa. However, the relative contributions of ecological selection, sexual selection, and drift to acoustic divergence have not typically been considered within the same study systems. The remainder of my dissertation used the Northern Hemisphere-distributed barn swallow (*Hirundo rustica*) species complex as a model system to study sender-receiver dynamics, intra- and intersexual selection pressures, and visual and acoustic signal interactions at the local scale, and signal divergence across populations at the global scale. From song recordings taken across 19 sampling sites, spanning five of six described subspecies, I demonstrated considerable conservation in song structure. However, temporal traits were highly divergent across subspecies, and in particular, the speed of the terminal trill of songs. In a detailed study of the multimodal communication system of the barn swallow (including visual and acoustic traits), I demonstrated that males and

females use different types of signals to mediate competition and mate choice. One of the only exceptions to this rule was trill rate, which was also implicated in song divergence across populations. In order to test the function of trill rate in communication, I performed a two-year playback study within the North American subspecies, *H. r. erythrogaster*. Contrary to expectations, males did not have stronger responses to faster trilling (high performance) simulated intruders. Instead, resident males had stronger responses to the high performance stimulus only when the intruder was also darker than the resident. Collectively, my dissertation offers novel insight into the evolutionary dynamics of complex sexual signaling at multiple spatial scales.

ACKNOWLEDGEMENTS

Undertaking my graduate research represents one of the great challenges of my life, and I am indebted to a large number of people for helping me over the course of the last six years. First and foremost, my graduate advisor, Rebecca Safran, has been an exemplary mentor, offering guidance, friendship, and support throughout my PhD. She provided me with attentive feedback at all stages of my scientific development, from the first formulation of my research ideas, to adding the finishing touches to manuscript revisions. She has also provided endless motivation through her massive enthusiasm for science and helped me accomplish things I would never have predicted possible during my PhD. But more importantly, Becca has created a family atmosphere in her lab, and I know I have gained lifelong friends in my advisor, labmates, and their families.

I would also like to thank my committee members, Deane Bowers, Mike Breed, Andy Martin, and Dan Mennill for their support of my research. In particular, I would like to thank Deane for keeping me in line and giving me a hug sometimes when I really needed it. To Mike Breed, I am thankful for what seemed to me an unearned, but unshakeable trust in my abilities which greatly helped my confidence as a graduate student. I thank Andy Martin for channeling Bill Murray—sometimes intimidating me with curveball questions, but always encouraging my creativity and breadth of interests with humor and wit. I thank Dan Mennill for taking substantial time out of his busy schedule to train me in how to record and analyze birdsong. He also made me feel at home in his lab at the University of Windsor, and I feel lucky to be a part of two lab families in the US and

Canada. Last, but not least, I thank two previous members of my graduate committee, Rob Guralnick and Patrik Nosil, for their early and ongoing support. You have both provided me invaluable advice and encouragement throughout the years.

In the past six years, I had the privilege to meet and interact with a huge number of undergraduates, graduate students, postdocs, faculty, and staff at the University of Colorado that have brightened my life in innumerable ways. I do not have space here to list them all, but I feel very privileged to have been part of the EBIO Department at CU, as it is a wonderfully open, collaborative, and fun community that I will miss (though I will keep in touch.) To members of my lab—you are amazing: Cait Dmitriew, Roi Dor, Andrew Flynn, Joey Hubbard, Amanda Hund, Brittany Jenkins, Liz Scordato, Maren Vitousek, and Rachel Bradley *nee* Wildrick. I would also like to name a few graduate students who have been especially important to me as friends, colleagues, and collaborators: Aaron Comeault, Chelsea Cook, Andrew Flynn, Joey Hubbard, Amanda Hund, Brittany Jenkins, Max Joseph, Nate Kleist, Jordan Kueneman, Helen McCreery, Joe Mihaljevic, Se Jin Song, and David Zonana. To my friends and housemates outside the department, thanks for keeping it real: Bella Alongi, Matt "Bruce" Cowan, Diana Vilar, Fabiola Vilar Rodriguez! This work would also have been impossible without a large number of collaborators with whom I feel privileged to work: Alex Buerkle, Shih-Fan Chan, Clint Francis, Keith Hobson, Joey Hubbard, Max Joseph, Martin Merz, Leyla Özkan, Peter Pap, Tom Parchman, Alex Rubtsov, Becca Safran, Liz Scordato, Nat Seddon, Georgy Semenov, Sheng-Feng Shen, Steven Van

Wilgenburg, Yoni Vortman, and especially Hakan Karaardıç, whose collaborative efforts and friendship have been extremely important to my success in grad school! This research also required the work of a huge number of field assistants: Matt Aberle, Stephen Alderfer, Rachel Bradley, Lauren Brooks, Özgün Eker, Connor Fitzhugh, Andrew Flynn, Michelle Foreman, Lori Fraser, Caroline Glidden, Ian Harold, Abdul Hussein, Leyla Kaplan, Martin Merz, Allison Mitchell, Alex Oesterle, Audrey Tobin, and Courtney Van Der Linden. In addition, Kate Zator provided invaluable help with song analysis.

Finally, without the loving support of my family, I would not have been set on a career path in biology, and would certainly not be the person I am today. I am forever grateful to my dad and stepmom, Jeff Wilkins and Kathy Williams, my mom and stepdad, Mary Jim and Keith Ailor, and my grandparents, Jim and Faye Reed and Carolyn Waldrep for their faith in me and for encouraging me to follow my heart. For being generally awesome, I thank my sisters Sarah and Anna Wilkins, and my extended family: Donna Payne and Jennifer Wilburn; Jane, Neil, Heath, and Ian Ross; and Wanda Ailor. Y'all are the best! Also, though he can't read, I would be remiss not to mention my amazing dog, Atticus, without whom I would've been a complete stressball during my PhD.

My dissertation was funded by University of Colorado grants from the EBIO Department, the Beverly Sears Fund, as well as undergraduate assistantship grants through the Biological Undergraduate Research & Skills Training (BURST) and Undergraduate Research Opportunities (UROP) Programs. Outside funding came from the Animal Behavior Society, Wilson Ornithological Society, American

Ornithologists' Union, Cooper Ornithological Society, as well as a Graduate Research Fellowship and a National Evolutionary Synthesis Center Semester Fellowship from the National Science Foundation.

CONTENTS

1 INTRODUCTION..... 1

2 EVOLUTIONARY DIVERGENCE IN ACOUSTIC SIGNALS: CAUSES AND CONSEQUENCES

2.1 Abstract..... 8
2.2 Introduction..... 9
2.3 Limits on Acoustic Divergence..... 11
2.4 The Acoustic Window Concept..... 12
2.5 Adaptive and Neutral Mechanisms of Divergence..... 15
2.6 Identifying Mechanisms Driving Acoustic Divergence..... 24
2.7 Current Knowledge on the Causes of Acoustic Divergence..... 24
2.8 Timing of Acoustic Divergence and Consequences for Speciation..... 29
2.9 The Role of Learning on Acoustic Divergence..... 32
2.10 Suggestions for Future Research..... 34
2.11 Conclusions..... 36

3 MULTIMODAL COMMUNICATION IN THE NORTH AMERICAN BARN SWALLOW: INFLUENCES OF INTRA- AND INTERSEXUAL SELECTION ON SIGNAL EVOLUTION

3.1 Abstract..... 37
3.2 Introduction..... 38
3.3 Methods..... 42
3.4 Results..... 54
3.5 Discussion..... 63
3.6 Conclusions..... 71

4 SONG DIVERGENCE ACROSS THE BARN SWALLOW SPECIES COMPLEX

4.1 Abstract..... 73
4.2 Introduction..... 74
4.3 Methods..... 76
4.4 Results..... 85
4.5 Discussion..... 91

5 THE ROLE OF TRILL RATE IN BARN SWALLOW COMMUNICATION: MALE-MALE COMPETITION, REPRODUCTIVE SUCCESS, AND SIGNAL INTERACTIONS

5.1 Abstract..... 93

5.2 Introduction..... 94
5.3 Methods..... 97
5.4 Results..... 105
5.5 Discussion..... 110
5.6 Conclusion..... 113

6 SUMMARY AND CONCLUSIONS

6.1 Summary of key findings..... 115
6.2 Contributions to understanding evolutionary processes in the barn swallow study system..... 117
6.3 Implications for the study of signal divergence..... 119
6.4 Implications for the study of complex signal evolution..... 120
6.5 Future directions..... 121
6.6 Conclusions..... 122

7 REFERENCES..... 124

8 APPENDIX..... 153

CHAPTER 1

INTRODUCTION

Sexual selection, the process through which mate choice decisions and competition for mates lead to differential reproductive success among individuals, effects phenotypic change within and across populations among a broad diversity of animals (Darwin 1871; Andersson 1994; Andersson and Simmons 2006). Following a seminal paper by Mary Jane West-Eberhard (1983), a great deal of research has focused on understanding the relative contributions of divergent ecological versus sexual selection to population divergence, and determining whether divergent sexual selection alone can lead to speciation (Price 1998; Higashi et al. 1999; Gray and Cade 2000; Boughman 2001; Panhuis et al. 2001; Masta and Maddison 2002; Stuart-Fox and Owens 2003; Seddon et al. 2008; Sauer and Hausdorf 2009; Arnegard et al. 2010; Seddon et al. 2013). This research has proved controversial, however (Ritchie 2007), with overall tentative support for a primary role of sexual selection during early divergence (Kraaijeveld et al. 2011), and theoretical models suggesting that ecological and sexual selection may often interact in the formation of new species (van Doorn et al. 2009; Bonduriansky 2011). Thus, a principal goal of my dissertation research was to improve our understanding of the causes and consequences of evolutionary divergence in sexual signals. For Chapter 2 of my dissertation, I established testable predictions for a role of sexual and natural selection, and interactions thereof, in acoustic divergence among closely related populations and performed a literature review, focusing on acoustic signals. The goal of this chapter was to synthesize our current knowledge on the roles of sexual

selection, ecological selection, and genetic drift in driving signal divergence, and the consequences of this divergence for speciation. Although I found support for sexual selection in driving acoustic divergence among the most well studied systems, all of these processes have not typically been considered within the same study organisms. Furthermore, a considerable amount of research effort has gone into the role of sensory drive in signal divergence. Sensory drive predicts that signals, sensory systems, and microhabitat choice coevolve as a function of natural and sexual selection (Endler 1992; Endler and Basolo 1998). Thus, previous work has demonstrated clear effects of habitat structure (Ryan and Brenowitz 1985; Ryan et al. 1990a; Patten et al. 2004; Braune et al. 2008; McNett and Cocroft 2008; Elias et al. 2010; Tobias et al. 2010), community composition (Zuk and Kolluru 1998; Chek et al. 2003; Kirschel et al. 2009a; Tinghitella and Zuk 2009; Grant and Grant 2010; Tobias et al. 2010), ambient noise profiles (Ryan and Brenowitz 1985; Kirschel et al. 2009a; Luther and Derryberry 2012), and sender/receiver physiology (Romer 1993; Podos 2001; DeVoogd 2004; Akre et al. 2011; Reinhold 2011; Derryberry et al. 2012) on the spectral and temporal properties of acoustic signals. However, the sensory drive hypothesis does not make specific predictions about the mechanisms responsible for these coevolutionary patterns. I offered the 'Acoustic Window' as an alternative conceptual framework for understanding how different selective regimes interact with evolutionary constraint to effect change in population signals over time. The acoustic window is defined as the acoustic parameter space which is available for signal evolution in a given taxon in a given habitat. As such, strong examples of sensory drive, such as adaptations of song to propagate effectively in

bamboo versus *terra firme* forest among Amazonian birds (Tobias et al. 2010), highlight transitions between acoustic windows, while most signal evolution may occur within acoustic windows.

The remainder of my dissertation research focused on understanding the processes underpinning the evolution of sexual signals within and divergence of signals across populations. To approach these questions, I used the barn swallow (*Hirundo rustica*) species complex, a migratory oscine songbird, which is distributed across the Northern Hemisphere and comprises six described subspecies (Dor et al. 2010; Turner 2010). As a classic model for sexual selection research, barn swallows have the advantage that much is known about many aspects of sexual selection in the nominate subspecies *H. r. rustica* in Europe (Møller 1988; Møller 1994; Galeotti et al. 1997; Saino et al. 1997a; Garamszegi et al. 2005; Garamszegi et al. 2006a; Møller et al. 2006). For example, it has long been known that sexual selection within this subspecies favors males with the longest tail feathers (streamers) (Møller 1988; Møller 1994).

However, a growing number of studies in other barn swallow populations show pronounced divergence in streamer length, ventral color, and associated preferences for these traits (Safran and McGraw 2004; Safran et al. 2005; Neuman et al. 2007; Hasegawa et al. 2010; Eikenaar et al. 2011; Vortman et al. 2011; Vortman et al. 2013). Song has only been quantitatively studied within Spanish and Italian populations of *H. r. rustica* (e.g. [Galeotti et al. 1997; Saino et al. 1997b; Møller et al. 1998; Garamszegi et al. 2006a]). Thus, for this system we have extensive baseline data for the nominate subspecies, growing evidence for divergent

sexual selection driving population differentiation, and a lack of characterization of song divergence across the species range. These attributes make barn swallows a good study system for furthering our understanding of how sexual selection pressures affect the evolution of communication across signaling modalities (i.e. acoustic and visual) and at various spatial scales.

Although a number of studies have quantified geographic variation in acoustic signals—e.g. for frogs (Prohl et al. 2006; Amézquita et al. 2009; Funk et al. 2009), arthropods (Henry and Wells 1990; Claridge and Morgan 1993; Shaw and Herlihy 2000a), birds (MacDougall-Shackleton and MacDougall-Shackleton 2001; Seddon 2005; Koetz et al. 2007; Podos and Warren 2007; Irwin et al. 2008; Liu et al. 2008; Sosa-López and Mennill 2014), and mammals (Delgado 2007; Braune et al. 2008; Cap et al. 2008; Campbell et al. 2010; Filatova et al. 2012)—it remains unclear whether random accumulation of differences over space (isolation by distance) or the effect of divergent selective pressures among populations (isolation by adaptation) provides a more general explanation of acoustic divergence. Thus, Chapter 5 of my dissertation, which I began first and finished last, provided the first description of geographic variation in barn swallow song. Sampling across 19 sites in six countries, and encompassing five of six described subspecies, I found remarkable conservation of the overall structure of barn swallow songs. However, there were pronounced differences in the temporal patterning of song components, and in particular the speed of pulse production in the terminal trill—termed "rattle tempo" in the literature (Garamszegi et al. 2006a). I also found that there was no pattern of isolation by distance, and variation in selection pressures among

populations (isolation by adaptation) likely provides a better explanation for geographic variation in song across this species' range.

In Chapter 3, I undertook a three year study on multimodal sexual selection pressures affecting the evolution of visual and acoustic signals in the North American barn swallow (*H. r. erythrogaster*). Recent work across diverse taxa has highlighted the importance of multimodal signals in mate choice and competition (Partan and Marler 1999; Candolin 2003; Partan 2004; Hebets and Papaj 2005; Partan and Marler 2005). However, for most systems, the relative roles of intrasexual selection (competition) versus intersexual selection (mate choice) in shaping communication systems is unknown (Wong and Candolin 2005). Thus, in this study, I assessed which aspects of color, morphology, and song were associated with surrogate measures of female choice and male-male competition. In addition, I applied tests of recent network theoretical predictions for the organization of signaling systems (Ay et al. 2007) to propose a new phenotype network-based approach for understanding complex signal evolution. Specifically, I tested whether the principle of robust over-design (Krakauer and Plotkin 2005) explains trait associations among signals utilized by different receivers in different contexts. Robust over-design predicts that signals within modalities should be tightly intercorrelated (redundant), while there should be weak associations across modalities to allow for a balance between robustness to the occlusion of any particular signal, and allowing for independent information to be conveyed by each modality. Overall, I found that there was minimal overlap between signals mediating male-male competition and female choice. A primary exception was trill

rate, which was also one of the most divergent song traits among populations. Moreover, I found that robust over-design explained the associations between traits involved in female choice, while the same was not true for traits mediating male-male competition.

Because trill rate was implicated in song divergence at the global scale and in both male-male competition and female choice within a population of *H. r. erythrogaster*, for Chapter 4, I performed a two-year playback study testing the role of this trait in communication. The goal of this study was to test the responses of territorial males to variation in trill rate to inform our understanding of sexual selection for this trait at local and global scales. For experimental design, I used taxidermic mounts paired with song stimuli to simulate territorial intrusions, and collected color, morphometric, and reproductive data for each resident male tested in the study. Previous work has shown that male swamp sparrows modulate responses to intruders varying in trill rate according to their own intrinsic trill rate (Moseley et al. 2013). In contrast, I found that males' intrinsic trill rate did not predict their latency (delay time) to respond to a high performance (fast-trilling) stimulus, a normal stimulus, or the difference of these values. Instead, resident males had shorter latencies to respond to a high performance stimulus only when the intruder male was darker than the resident. Moreover, males which had higher latencies to respond to a high performance than a normal stimulus had a larger number of offspring survive to fledging. Interestingly, difference in latencies across high performance and normal experimental treatments did not predict the number of genetic offspring in residents' nests.

Collectively, this dissertation offers insight into the patterns and processes relating to the evolution of sexual signals at various scales. I have provided a synthesis of the causes and consequences of acoustic divergence across diverse animal taxa, and offered the acoustic window concept as a new approach for incorporating stochastic and deterministic processes, and evolutionary constraint into investigations of signal divergence and speciation. I have also provided the first description of geographic variation in barn swallow song across the species range, demonstrating pronounced divergence in temporal characteristics, with minimal evolution in song structure, and no evidence for isolation by distance in signal differentiation. Through my investigations of multimodal sexual selection and the role of trill rate in barn swallow communication, I showed that while there may be little overlap in the signals involved in intra- versus intersexual selection, the traits which are utilized in both contexts may be of particular import. Moreover, the results of my trill experiment highlight the importance of understanding how multimodal signal interactions affect receiver behavior. I hope this work will provide the foundation for much future research on the evolution of complex signals and its interaction with the process of speciation.

CHAPTER 2

EVOLUTIONARY DIVERGENCE IN ACOUSTIC SIGNALS: CAUSES AND CONSEQUENCES [1]

2.1 Abstract

Acoustic signals mediate mate choice, resource defense, and species recognition in a broad range of taxa. It has been proposed, therefore, that divergence in acoustic signals plays a key role in speciation. Nonetheless, the processes driving divergence of acoustic traits and their consequences in terms of speciation are poorly understood. A review of empirical and comparative studies reveals strong support for a role of sexual selection in acoustic divergence, but the possible concomitant influences of ecological context are rarely examined. We summarize a conceptual framework for testing the relative significance of both adaptive and neutral mechanisms leading to acoustic divergence, predictions for cases where these processes lead to speciation, and how their relative importance plays out over evolutionary time.

---

[1] Published as: Wilkins, MR, Seddon, N, Safran, RJ (2013) *Trends in Ecology and Evolution*, **28** (3): 156-166.

2.2 Introduction

In taxa as diverse as frogs, insects, mammals, birds, and to an underappreciated extent, spiders and fish, acoustic signals function in mate choice, resource defense, and species recognition (Marler and Slabbekoorn 2004). Unlike fine-scale features of morphology or other signals that require the close proximity of receivers, acoustic signals can be detected at a distance. Moreover, these signals often concurrently encode information about the signaler's identity, location, and condition, thereby reducing the costs associated with direct encounters. Acoustic signals are therefore especially suited to mediate discrimination within and between species (Mendelson and Shaw 2012). Additionally, the simple genetic architecture of some acoustic signals (Shaw et al. 2011) or cultural mutations gained in the acquisition of learned acoustic traits (Lachlan and Servedio 2004), allow for rapid changes in signal structure which can facilitate divergence. Given these features, it is not surprising that acoustic signals often distinguish recently diverged taxa better than other phenotypic traits (Henry 1994; Mendelson and Shaw 2005; Toews and Irwin 2008; Funk et al. 2012). Indeed, rapidly speciating lineages are often only identified by differences in acoustic signals (e.g. cicadas (Marshall et al. 2008), swordtail crickets (Mendelson and Shaw 2005), and green lacewings (Henry 1994)), and playback experiments in many systems demonstrate that signal divergence effects species recognition (see glossary in Appendix 8A2.1) and mate choice (Irwin et al. 2001b; Braune et al. 2008; Noh and Henry 2010; Parker et al. 2010; Podos 2010; Seddon and Tobias 2010). Moreover, phylogenetic comparative studies reveal lineage-specific acoustic differences (Mendelson and Shaw 2005; Marshall et al. 2008) and show that

divergence in acoustic traits predicts patterns of diversification across genera (Seddon et al. 2008). Together these findings indicate a key role for acoustic signals in the diversification of species – either early or late in the process – in a broad range of organisms.

Despite accumulating evidence for a major role of acoustic signals in driving and/or finalizing species divergence, several fundamental questions remain. In particular, the importance of adaptive versus neutral processes in acoustic divergence is unknown in most studies, and there is currently no clear hypothesis-testing framework to differentiate the relative significance of drivers of acoustic divergence. Additionally, it is currently unclear whether acoustic divergence is more important in facilitating speciation by providing a pre-mating barrier early (where there is little genetic and no morphological divergence between taxa) (Henry 1994; Mendelson and Shaw 2005) or later in the speciation process (i.e. during secondary sympatry, where genetic divergence has led to Dobzhansky-Muller incompatibilities between taxa) (Noor and Feder 2006).

Here we begin by discussing factors which limit standing variation in acoustic traits and potential evolutionary outcomes of acoustic divergence (Section 2.3, Table 2.1). We then: 1) summarize current knowledge and review support for the processes leading to acoustic divergence (Section 2.5, Table 2.22); 2) propose guidelines for testing hypotheses about neutral and adaptive processes in driving acoustic divergence (adaptive and neutral processes in Section 2.6, Figure 2.11 Table 2.2); 3) provide a summary of current knowledge related to the causes and consequences of acoustic divergence (Section 2.7, Table 2.3); and 4) offer predictions for testing the

timeframe over which acoustic divergence initiates or finalizes speciation (Section 2.8). We conclude by suggesting methods and lines of inquiry most likely to provide key insights into outstanding questions at the interface of acoustic signaling and speciation (Section 2.10).

2.3 Limits on Acoustic Divergence

Table 2.1 summarizes the major factors determining the parameter space, or "acoustic window," within which acoustic signals can evolve. Much work attempting to understand these factors has focused on testing the Acoustic Adaptation Hypothesis (Morton 1975) or the broader Sensory Drive Framework, a framework devised by Endler (Endler 1992) for the process by which signals and sensory systems coevolve as a function of habitat structure, ambient noise, presence of predators, and other sensory considerations. While much evidence for sensory drive comes from visually communicating systems (Seehausen et al. 2008), its role in shaping acoustic communication is less well supported and most studies have focused on the effects of habitat on patterns of signal divergence. This work has demonstrated clear effects of habitat structure (Ryan and Brenowitz 1985; Ryan et al. 1990a; Patten et al. 2004; Braune et al. 2008; McNett and Cocroft 2008; Elias et al. 2010; Tobias et al. 2010), community composition (Zuk and Kolluru 1998; Chek et al. 2003; Kirschel et al. 2009a; Tinghitella and Zuk 2009; Grant and Grant 2010; Tobias et al. 2010), ambient noise profiles (Ryan and Brenowitz 1985; Kirschel et al. 2009a; Luther and Derryberry 2012), and sender/receiver physiology (Romer 1993; Podos 2001; DeVoogd 2004; Akre et al. 2011; Reinhold 2011; Derryberry et al. 2012) on the spectral and temporal properties of acoustic signals. These studies show a match between signal

variation and measures of optimal signal transmission. For example, divergence between Amazonian bamboo-specialist bird species and their nearest relatives in *terra firme* forest correlates with habitat sound transmission properties, rather than genetic distance, ambient noise, or mass (Tobias et al. 2010). However, we stress that the sensory drive framework itself is not a mechanistic explanation of evolutionary change responsible for signal divergence (Section 2.9). Rather, we suggest that this approach delimits the amount of standing acoustic variation available for ecological selection, sexual selection, and drift in a given habitat.

2.4 The Acoustic Window Concept

For a given population, aspects of habitat structure, ambient noise, presence of parasitoids and predators, and the neurophysiology of senders and receivers will determine the effective "acoustic window" available for evolutionary change in signals (see figure below). Changes in any of these factors (e.g. movement into a new habitat) will affect the acoustic window, truncating variation available for adaptive or neutral evolution.

A recent meta-analysis in birds showed that habitat structure (coarsely defined as open versus closed) had a significant effect on peak frequency, but much weaker or nonsignificant effects on other spectral traits and interval duration (Boncoraglio and Saino 2007). Thus, for birds, limits on audible signal variation imposed by habitat structure lead to predictable effects on song pitch via sensory drive, while much of the differentiation among populations must be due to other adaptive and neutral processes.

Table 2.1. Major constraints affecting acoustic signal evolution.

Factor	Effect	Examples	Refs
Physical features of habitat	Limits standing variation	Emphasized frequencies (i.e. pitch) of vocalizations have been shown to match those expected for optimization of signal transmission (i.e. minimize attenuation and reverberation) in a given habitat for some passerine birds, insects, spiders, frogs and mammals.	(Ryan and Brenowitz 1985; Ryan et al. 1990a; Patten et al. 2004; Braune et al. 2008; McNett and Cocroft 2008; Elias et al. 2010; Tobias et al. 2010)
Community composition		The presence of community members that produce acoustic signals may lead to strong divergent selection on signal structure to avoid masking interference, as shown in frogs and birds. Presence of predators or parasitoids may also select for reduced signal elaboration, or signal loss.	(Zuk and Kolluru 1998; Chek et al. 2003; Kirschel et al. 2009a; Tinghitella and Zuk 2009; Grant and Grant 2010; Tobias et al. 2010)
Ambient noise		Certain taxa may be excluded from a habitat, or will adapt signal structure to avoid masking interference by biotic and abiotic sources of ambient noise.	(Ryan and Brenowitz 1985; Kirschel et al. 2009a; Francis et al. 2011; Luther and Derryberry 2012)
Phylogenetic history		Shared derived or ancestral traits (e.g. body size or beak morphology) evolved in other social or ecological contexts may limit the variation available for signal evolution.	(Ryan and Brenowitz 1985; Romer 1993; Podos 2001)
Sender morphology and neurophysiology	Limits potential evolutionary outcomes	Acoustic signals are often constrained by morphological (e.g. beak shape or body size) and neurological limits.	(Romer 1993; Podos 2001; DeVoogd 2004; Derryberry et al. 2012)
Receiver morphology and neurophysiology		Receiver morphology (e.g. hearing structures) and neurophysiology (i.e. the neurological structures affecting perceptual and cognitive abilities) may restrict elaboration by signalers.	(Romer 1993; Akre et al. 2011; Reinhold 2011)

As illustrated by the dashed arrow in the figure below, sensory drive only has explanatory value where the ancestral and novel acoustic windows do not overlap. Sensory drive will explain little of the acoustic signal variation among taxa which are capable of a wide variety of sounds, adopt behavioral mechanisms to avoid heterospecific interference, inhabit environments with high signal propagation, or have reduced selection by acoustically orienting predators.

Examples supporting sensory drive highlight taxa with narrow and non-overlapping acoustic windows among populations and could over-represent the general importance of constraints on acoustic divergence. The relatively low effect of sensory drive shown in birds (Boncoraglio and Saino 2007) suggests that signal divergence within an acoustic window (solid arrow, below) might be the more common scenario. Other factors not generally considered in sensory drive, such as phylogenetic history, could also limit signal evolution. Previous adaptations (e.g. body size or beak morphology) (Ryan and Brenowitz 1985; Romer 1993; Podos 2001) evolved in other social or ecological contexts can impose limits on how signals can respond to selection. Moreover, physiological tradeoffs might result in holes in the multidimensional parameter space of the acoustic window (e.g. trill rate only increases at the expense of frequency bandwidth) (Podos 2001; Derryberry et al. 2012).

Thus, identifying factors which define the acoustic window and determine the possible directions for signal evolution within it allows for a clear understanding of how constraint affects signal distributions available to selection and drift. However, in order to better understand the mechanism by which signal distributions move into

and within an acoustic window, the contributions of neutral and adaptive processes should be considered directly (Table 2.2).

2.5 Adaptive and neutral mechanisms of divergence

In this review we consider ecological and sexual selection separately and outline testable predictions for determining the relative contribution of each of these processes to adaptive acoustic signal divergence (Table 2.2).

2.5.1 Ecological selection

Sources of acoustic divergence

If acoustic differences between populations result *primarily* from divergent ecological selection, ecological trait divergence is predicted to co-vary with the degree of acoustic differentiation between sister taxa, while strength of preference for local variants or strength of sexual selection does not (Table 2.2). Here, we discuss cases where ecological selection was likely the dominant driver of signal divergence.

In bats, disruptive ecological selection can commonly act on echolocation frequency, in association with specialization on different prey size classes (Jones 1997). In this way direct ecological selection on signal function leads to acoustic divergence. Perhaps a more common scenario results from correlated ecological selection on morphological traits involved in sound production. For example, differentiation of beak size for efficient foraging on seeds has had a pleiotropic effect on song trill rate in Galápagos finches: birds with larger bills are only able to produce slow-paced trills (Podos 2001).

Table 2.2 Testable predictions for signal divergence under four different selection regimes: ecological selection, sexual selection, a combination, or drift (absence of selection).

	General expected patterns under each selection regime				Testable predictions for empirical studies		
Selection regime (i.e. primary contributor to acoustic divergence)	Acoustic signal variation within populations covaries with:	Acoustic signal divergence among populations covaries with:	Mate selection pattern (assortative or preference-based*)	Population-level acoustic trait variation	Field-based comparative study: what is the relative contribution of ecological and sexual selection in the evolution of acoustic divergence?	Phylogenetically controlled correlated trait evolution: is acoustic signal evolution correlated with ecological selection, sexual selection or both?	Experimental evolution study: what causes acoustic divergence between replicated lines?
Ecological Selection	Survivorship or ecological performance, as a result of direct (e.g. on bat echolocation call) or correlated selection (e.g. finch beak size).	Ecological divergence (e.g. differences in beak depth, body size), features of the environment (e.g. climatic variables, signal transmission properties), or ecological performance (e.g. capture of certain prey sizes by echolocation).	Assortative	Low; signals do not function as quality indicators, but may instead serve as recognition cues, with greater trait variation between than within populations	Within separate populations, acoustic signals co-vary with ecological traits including morphological features related to signal production. Acoustic divergence occurs primarily as a function of environmental divergence (e.g. diet, predators, parasites, acoustic environment) and corresponds with assortative mating.	Divergence in ecological traits (e.g. beak size or wing length in birds) predicts acoustic divergence.	Population signals converge in a common garden setting within replicated lines.

Sexual selection	Reproductive success, strength of mate preference or level of intrasexual competition.	Divergence in the intensity of sexual selection or strength of preference for local signal.	Preference-based	Relatively high; acoustic signals serve as quality indicators	Within separate populations, acoustic signals covary with intensity of sexual selection, controlling for ecological differences. Signal divergence is driven by divergent preferences such that individuals show greatest response to the most exaggerated form of the local signal.	Divergence in sexual signaling traits (e.g. degree of sexual dimorphism) or intensity of sexual selection (e.g. degree of polygyny) predicts acoustic divergence.	Population signals diverge or remain constant in a common garden setting within replicated lines.
Ecological and Sexual Selection	Divergence in both ecological and sexual traits/ intensity of sexual selection.	Divergence in both ecological variables and intensity of sexual selection between closely related populations.	Preference-based	Relatively high; acoustic signals are indicator traits and vary among individuals	Within separate populations, acoustic signals covary with both ecological and sexual traits/ intensity of sexual selection. Individuals show greatest response to the most exaggerated form of the local signal.	Divergence in both ecological traits and sexual traits predicts acoustic divergence.	Population signals converge to maximize signal efficacy, while stochastic targets of sexual selection may lead to increased divergence in some signal features among replicated lines.
Drift	Divergence in neither ecological nor sexual traits/ intensity of sexual selection.	Divergence in neither ecological variables nor intensity of sexual selection between closely related populations.	Random mating with regard to acoustic signals	No specific predictions	Acoustic signals do not covary with ecological or sexual traits/ intensity of sexual selection and individuals do not show greatest response to any particular variant of the local signal. Acoustic distance accrues linearly with neutral genetic distance.	Acoustic divergence is not associated with ecological or sexual trait divergence.	Population signals vary stochastically across replicates.

*See Glossary for definitions.

Another potential source of divergent ecological selection that impacts acoustic signals relates to body size. There is a general tendency for larger animals to have lower pitched acoustic signals (Ryan and Brenowitz 1985; Seddon 2005; Gillooly and Ophir 2010). This relationship results from a positive correlation between body size and the mass of vibratory structures related to sound production: the larger the structures the lower pitch of the sounds produced (Ryan and Brenowitz 1985). Even more generally, body mass explains much of the variation in signal pitch and duration across the major acoustically signaling animal lineages (Gillooly and Ophir 2010). As a result, selection on signal pitch can lead to inverse correlated effects on body size and vice versa, as shown in frogs (Hoskin et al. 2005; Boul et al. 2007). Evolution of acoustic traits resulting from ecological adaptation might be widespread, due to the generality of the size-pitch relationship across taxa.

Consequences of acoustic divergence

To demonstrate that speciation results primarily from ecologically selected acoustic divergence, studies should show that divergence in signals 1) corresponds with ecologically adaptive trait variation (directly or through trait correlation) in each population and 2) is associated with assortative mating (see Appendix 2.1 for glossary) by ecotype. For example, ecologically selected changes in echolocation frequency among Wallacea's bats affect sensory perception of prey items. Three discrete size morphs thus utilize echolocation frequencies at 53.6 kHz, 39 kHz, and 27 kHz, to better detect small, medium, and large insects, respectively. Increased sensitivities at these call pitches is also thought to cause pre-mating isolation through effects on mating signals and perception (Kingston and Rossiter 2004). Similarly, a

population of the medium ground finch of El Garrapatero, Galápagos has experienced disruptive selection for beak size. Because of correlated bioacoustic effects of these adaptations, small-beaked morphs have broader frequency bandwidth songs, and both morphs respond more strongly to homotypic (i.e. local) song (Podos 2010). Although acoustic divergence in bats results from direct selection on signal function, and divergence in finches results from correlated selection on beak size, both traits are considered 'magic traits', because ecologically adaptive changes in these traits results in assortative mating (reviewed in Servedio et al. 2011). Magic traits provide an important starting point for studying the interplay between ecological and sexual selection. However, magic trait studies have typically assumed a pattern of assortative mating resulting as a byproduct of ecological divergence, without investigating the possibility of preference-based mating through sexual selection (see glossary). Characterization of sexual selection pressures in these systems may validate this assumption or reveal a more dynamic interplay between sexual and ecological selection.

2.5.2 Sexual Selection

Sources of acoustic divergence

If sexual selection has been the dominant source of selection responsible for acoustic divergence, strength of preference for, or intrasexual aggression elicited by (Tobias et al. 2011), local acoustic signals should be a stronger predictor of acoustic divergence than differentiation in ecological traits. A key prediction of this model is that ecological differences play little or no role in shaping patterns of acoustic signal divergence. Sexual selection has been proposed as the primary driver of acoustic

divergence between populations in a diversity of taxa, including frogs (Boul et al. 2007), green lacewings (Henry et al. 2002), crickets (Gray and Cade 2000; Shaw and Lesnick 2009), and birds (Irwin et al. 2001b; Toews and Irwin 2008). For example, female preference for greater signal complexity is thought to have driven acoustic divergence between two species of winter wren (Toews and Irwin 2008), subspecies of greenish warbler (Irwin et al. 2001b), and some populations of Peters' dwarf frog (Boul et al. 2007). However, the mechanisms by which preferences diverge in these systems are not well understood.

Consequences of acoustic divergence

To clearly demonstrate speciation via sexually selected acoustic divergence, studies should show that divergence in signals 1) corresponds with sexual selection pressures in each population and 2) is associated with divergent preferences. Usually, data are available for criterion 1 or 2, but not both (but see (Rodríguez et al. 2006)). Additionally, phylogenetic techniques have been employed to test whether patterns such as increased signal complexity, an expected product of sexual selection, could explain patterns of species diversity (Mendelson and Shaw 2005; Boul et al. 2007; Seddon et al. 2008). The best example we are aware of, which combines all of these levels of inquiry, is the radiation of the Hawaiian swordtail crickets (*Laupala spp.*). In this system, phylogenetic methods have shown that genetic differences between species are associated with differences in call pulse rate (Mendelson and Shaw 2005). Moreover, experimental work within divergent populations of *Laupala cerasina* has shown that 1) females prefer mean values of local male pulse rates, and 2) these differences result in preferences for local mates, establishing a pre-mating barrier

(Grace and Shaw 2012). Thus, acoustic divergence, initiated by divergent sexual selection, seems to have occurred without apparent ecological adaptation (Mendelson and Shaw 2005).

2.5.3 Ecological Selection + Sexual Selection Model

Theory suggests that ecological adaptation and mechanisms of mate choice are closely entwined, and can be mutually reinforcing (van Doorn et al. 2009; Bonduriansky 2011; Maan and Seehausen 2011). In particular, sexual traits and preferences will always be expressed within an ecological context and might therefore be subject to ecological selection (Maan and Seehausen 2011). It has also been suggested that sexual selection might accelerate population divergence initiated by disruptive ecological selection (van Doorn et al. 2009) and potentially facilitate ecological adaptation by displacing populations from optimal viability peaks (Bonduriansky 2011).

Sources of acoustic divergence

If ecological and sexual selection acting in combination are largely responsible for signal divergence, acoustic differences among populations should covary with divergence in both ecological variables and sexual traits. One of very few systems where data are available on both of these sources of selection is the *Enchenopa binotata* treehopper complex. In this complex, host plant shifts are associated with ecologically-selected changes in signal pitch, corresponding with optimal signal transmission through plant substrates (McNett and Cocroft 2008). Thus, the acoustic window for each host plant habitat is limited to a narrow frequency band. Further signal evolution within these bounds results from sexual selection by female choice

(Sullivan-Beckers and Cocroft 2010). Additional examples from cricket frogs and song sparrows show correlations between sexually-selected acoustic traits and body size and parasite load, respectively (Table 2.2).

Consequences of acoustic divergence

For speciation to result from divergent ecological and sexual selection on acoustic signals, studies should show that divergence in signals 1) covaries with ecologically adaptive trait variation and 2) sexual selection pressures in each population, and 3) is associated with divergent preferences. Treehoppers are the only study system we are aware of which satisfy these criteria. It has been shown that signal variation corresponds with ecologically adaptive transmission properties based on signaling substrate (McNett and Cocroft 2008), signals across populations correspond to strength of female preference (Rodríguez et al. 2006), and local signals are preferred, resulting in reproductive isolation (Sullivan-Beckers and Cocroft 2010).

2.5.4 Genetic Drift, Cultural Drift, and Mutation-Order Processes

Sources of acoustic divergence

In addition to adaptive drivers, selectively neutral changes between populations resulting from differential sampling of alleles involved in sound production can also lead to acoustic divergence. To demonstrate that neutral evolution is most important in driving acoustic divergence, studies should invalidate criteria for each potential selection regime (Table 2.2), and show that acoustic trait divergence increases linearly with genetic or geographic distance (Figure 2.2, blue dashed line). The latter criterion has been fulfilled for greenish warblers (Irwin et al. 2008), frogs (Amézquita et al. 2009), and singing mice (Campbell et al. 2010), where it was attributed to drift.

(For animals with vocal learning (Section 2.9) such as oscine passerines, cultural and genetic drift will be difficult to separate.) However, it was also recently suggested that a linear accumulation of acoustic differences over time could result from a mutation-order (M-O) process (Martin and Mendelson 2012). If populations adapting to similar environments randomly gain beneficial (but incompatible) mutations in a clock-like fashion, and there are minor fitness differences between alleles, this can lead to M-O speciation. Although drift should be more important in smaller population sizes, the interaction between drift and M-O processes merits further investigation (Nosil and Flaxman 2011). Thus, demonstrating the first criterion, that selection has had a minor effect on acoustic divergence, is not trivial. In many cases, such as in greenish warblers, acoustic divergence may occur through a combination of selection and drift (Irwin et al. 2008).

Consequences of acoustic divergence

Unlike differences resulting from deterministic processes, there is no clear expectation that acoustic divergence through drift will result in a pre-mating barrier. To demonstrate that speciation results from acoustic divergence by drift, studies should show that: 1) acoustic signals have evolved through drift, with little effect of selection, and 2) there is assortative mating by population. Drift will most likely lead to speciation where population demographics or time in allopatry allow for greatest divergence in signal, preference, or both.

2.6 Framework for identifying mechanisms driving acoustic divergence and speciation

Table 2.2 outlines a conceptual framework for determining the relative contributions of ecological, sexual selection, and drift to acoustic divergence. The patterns and study methodologies provided in table 2.2 allow one to determine which process(es) are largely responsible for acoustic divergence. Because one single methodology is not applicable to all study taxa, we also provide specific testable predictions for distinguishing between these primary sources of selection through a variety of approaches. Accordingly, this framework can be applied to empirical studies that examine two or more closely related populations, or to larger-scale phylogenetic comparative studies. Complementary studies utilizing preference tests or phylogenetic studies of diversification patterns should aim to verify that acoustic differences are related to reproductive isolation. Ideal systems for testing the role of sexual and ecological selection in acoustic signal divergence (i.e. birds, crickets, spiders, and bats) have a wealth of acoustic, ecological, and phylogenetic data available, as well as information on sexual selection pressures among populations. We believe that studies using this integrated approach will greatly benefit our understanding of the adaptive drivers of diversification.

2.7 Current knowledge on the causes and consequences of acoustic divergence

Table 2.3 summarizes the current state of knowledge on the role of different drivers of acoustic divergence in speciation for the best-studied taxa (see also supplementary table). Most studies have not attempted to disentangle the relative contributions of evolutionary constraints, adaptive processes, and neutral mechanisms in acoustic

divergence. Evidence that sensory drive plays a role in acoustic divergence comes mainly from birds (Ryan and Brenowitz 1985; Podos 2001; DeVoogd 2004; Patten et al. 2004; Kirschel et al. 2009a; Grant and Grant 2010; Tobias et al. 2010; Francis et al. 2011; Derryberry et al. 2012; Luther and Derryberry 2012), but also insects (Romer 1993; Zuk and Kolluru 1998; McNett and Cocroft 2008; Tinghitella and Zuk 2009; Reinhold 2011), spiders (Elias et al. 2010), frogs (Ryan et al. 1990a; Akre et al. 2011; Reinhold 2011), and mammals (Braune et al. 2008), suggesting the importance of selective shifts between acoustic windows. For example, host plant characteristics result in very narrow frequency bands (in the order of 100Hz) for optimal transmission of vibrational signals in treehoppers (McNett and Cocroft 2008). Thus, transmission properties severely limit acoustic windows in substrate-signaling treehoppers, while ambient noise may provide a greater limitation on the acoustic windows of aerially signaling birds (Ryan and Brenowitz 1985; Kirschel et al. 2009a; Luther and Derryberry 2012), whose signals commonly encompass more than 1000Hz. Moreover, habitat transmission properties might be important in affecting emphasized frequencies or frequency bandwidth, but not the fine-scale spectral characteristics or temporal patterning of birdsong (Boncoraglio and Saino 2007). Habitat is also generally less important in explaining variation in frog (Kime et al. 2000) and insect (Jain and Balakrishnan 2012) signals, perhaps due to larger effects of evolutionary constraint, available acoustic space, or the direction of sexual selection. A focus on sensory drive is important, but further resolution on signal divergence can be gained from testing the adaptive or neutral processes underlying transitions between and shaping variation within acoustic windows.

A role of sexual selection in acoustic divergence features prominently in the best-studied taxa. For example, of the nine taxa included in Table 2.3, chosen to represent a broad range of animal groups for which many factors influencing acoustic divergence have been considered, eight indicate an important role for sexual selection. A broader dataset of 18 taxa (table 8A2.2), including less well-studied groups, illustrates a similar pattern. We argue that these results represent a real trend, stemming from the general importance of acoustic signals in sexual communication, and the propensity for changes in these signals among populations to lead to speciation. This is consistent with a recent meta-analysis of comparative studies indicating a significant positive effect of sexual selection on speciation rates, though much depended on the depth of phylogenetic sampling (Kraaijeveld et al. 2011). However, for studies showing a role of sexual selection in acoustic divergence, we know very little about the contribution of ecological selection to signal variation.

2.8 The timing of acoustic divergence and its consequences for speciation

Although this question has not been systematically addressed for acoustically signaling organisms, we suggest that certain characteristics, discussed below, will make acoustic divergence more important early (initiating) versus later (finalizing) the speciation process. Figure 2.2 shows predicted relationships between acoustic and neutral genetic distance resulting from different processes over different timescales.

Table 2.3. Summary of the best-studied taxa for acoustics and speciation

			Constraints	Processes					
Common name ***Scientific name***	**Taxon**	**Level of comparison**	**Sensory drive tested?**	**Ecological Selection tested?**	**Sexual Selection tested?**	**Role of Drift tested?**	**Proposed driver of AD**	**Summary**	**Refs**
Peters' dwarf frog *Engystomops petersi*	Amphibian	Population	No	In part, tested correlation between signal and landscape features	Yes	Yes	SS	Sexual selection for call complexity has driven divergence in calls and associated structures; ecological selection (as tested) not important; sensory drive untested.	(Boul et al. 2007; Funk et al. 2009)
Cricket frog *Acris crepitans* (now *Acris blanchardi*)	Amphibian	Population	Yes	Body size only	Yes	No	SS	Females select for lower pitched signals, regardless of population identity; sensory drive explains salient differences between populations in different habitats.	(Ryan et al. 1990a; Ryan et al. 1992; Gamble et al. 2008)
Swordtail crickets *Laupala spp.*	Insect	Species	No	No ecological distinctions found	Yes	No	SS	Sexual selection for pulse rate has driven rapid divergence, without obvious ecological adaptation; role of drift unknown; sensory drive untested.	(Shaw and Herlihy 2000b; Mendelson and Shaw 2005; Grace and Shaw 2012)
Treehoppers *Enchenopa binotata*	Insect	Host races	Yes	Habitat categories (host plant)	Yes	No	ES + SS	Sexual selection following host shift strongly supported; ecological selection and drift not directly tested; sensory drive important.	(Sullivan-Beckers and Cocroft 2010)

Green lacewings *Chrysoperla spp.*	Insect	Songtype species	Yes	No	Yes	No	SS	Sexual selection proposed, but a relationship between signal variation and fitness not demonstrated. Mutation order speciation may be more likely; sensory drive not very important.	(Henry and Wells 2004; Noh and Henry 2010)
Amazonian birds	Suboscines and nonpasserines (i.e. no song learning)	Closest relatives in bamboo and *terra firme* habitat (congeners, but not sisters)	Yes	Yes	No, thought unlikely to be important	Yes	Unk	Sexual selection thought to be unlikely, and ecological traits not found to predict signal variation; sensory drive important in signal divergence.	(Tobias et al. 2010)
Song sparrow *Melospiza melodia*	Oscine passerine	Subspecies	Yes	Yes, through parasite loads	Yes	Yes	Unk	Sexual selection for locally common song elements supported; local song element sharing inversely correlated with parasite load; drift supported; sensory drive explains significant amount of signal variation.	(Patten et al. 2004; Stewart and MacDougall-Shackleton 2008)
Greenish warblers *Phylloscopus trochiloides*	Oscine passerine	Subspecies	Considered, not tested	No	Indirectly, through playbacks to males	Yes	Drift and SS	Sexual selection for complexity along northern gradient and drift proposed to explain song divergence; ecological selection untested; sensory drive untested.	(Irwin et al. 2001b; Irwin et al. 2008)
Medium ground finch *Geospiza fortis*	Oscine passerine	Populations	Yes	Yes	Indirectly, through playbacks to males	No	ES	Ecological selection for beak size results in population divergence through assortative mating; unknown whether song characteristics relate to mating success; sensory drive not well-supported; drift untested.	(Bowman 1979; Hendry et al. 2009; Podos 2010)
AD=Acoustic Divergence; ES= Ecological selection; SS=Sexual Selection; Unk=Unknown									

Early acoustic divergence

For acoustic signal divergence to provide the primary isolating barrier early in speciation, there should be changes in signals and/or perception, and these changes must be tightly coupled with species recognition and assortative mating. This process should be facilitated by reduced constraints on signal or perceptual evolution, controlled by simple genetic architecture or subject to rapid cultural evolution, especially where there is tight linkage between signal and preference (Shaw et al. 2011). The key expectation for the importance of acoustic divergence early in speciation is that signal differences will accumulate much faster than genetic differences. Thus, the best-fit function between genetic and acoustic divergence will have a non-zero intercept (Figure 2.2, green dashed line).

This pattern is likely to be especially important in organisms such as vibratory-signaling insects (e.g. lacewings and treehoppers), which are able to adapt spectral and/or temporal characteristics of signals through a simple mechanism that does not require large-scale changes in signaling organs (Henry and Wells 2004; Cocroft et al. 2010). Additionally, organisms with tight linkage of signal and preference (e.g. swordtail crickets) (Shaw and Lesnick 2009) might be predisposed to speciation via early acoustic divergence.

Such rapid speciation should primarily occur through two processes. The first involves magic traits, which have traditionally been studied in the context of host-plant specialization. However, a new study in birds (Derryberry et al. 2012) highlights their relevance to speciation through acoustic divergence, as selection on

beak size can have correlated effects on song production, leading to reproductive isolation. In the second process, M-O speciation, the mutation is related to acoustic signal production, and species recognition, but is selectively equivalent among populations from an ecological standpoint.

Perhaps the best example of this process is found in the green lacewing radiation. These duetting insects appear to speciate readily through simple mutations which directly cause assortative mating through effects on the signals of males and females (Henry 1994). Although sexual selection might affect later signal

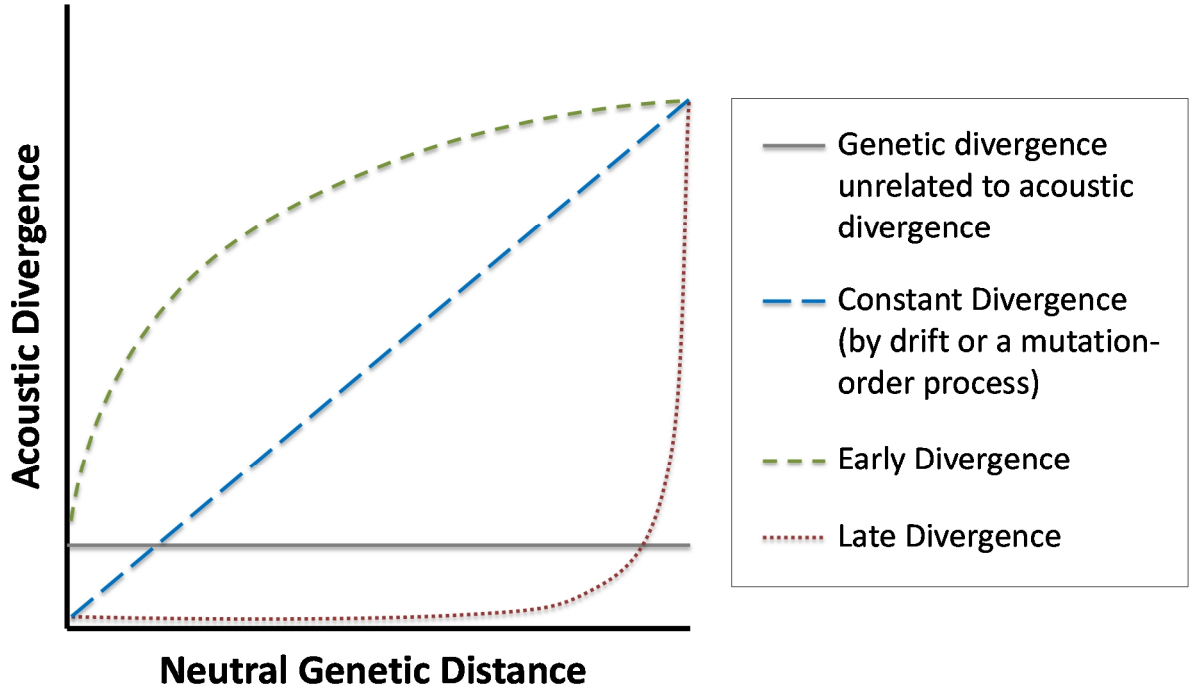

Figure 2.2 Predictions for comparisons of acoustic and genetic divergence across populations, which are expected to result from different processes. Predicted patterns are as follows: there is no relationship between acoustic and genetic divergence, perhaps due to context-specific environmental or demographic factors (gray, solid line); acoustic and neutral genetic distances accrue in a clocklike fashion, as expected through drift or a mutation-order process (blue, long-dashed line); acoustic signals diverge faster than neutral genetic loci, as a result of ecological, sexual selection, or a combination (green, short-dashed line); or acoustic signals diverge slowly until neutral genetic differences have accrued, and then rapidly increase, as expected by reinforcement (red, dotted line). The green, short-dashed line is consistent with a role for acoustic divergence in initiating speciation in the lineage under study, while the red, dotted line is consistent with the importance of acoustic divergence in finalizing speciation. This predictive framework is amenable to comparative studies.

evolution, the fact that species recognition is based on a simple mutation, which does not appear to have adaptive transmission properties related to habitat choice (Henry and Wells 2004), makes this system a good candidate for M-O speciation.

Acoustic divergence later in speciation: a role of reproductive character displacement

Great effort has been spent investigating the effects of secondary contact between divergent taxa on acoustic divergence. Once post-zygotic genetic incompatibilities have arisen, selection should favor increased divergence in signals and their discrimination. Such a pattern of reproductive character displacement (RCD) has been demonstrated in a wide range of taxa, including insects (Marshall and Cooley 2000), frogs (Hoskin et al. 2005), bats (Jones and Siemers 2011), and birds (Seddon 2005; Kirschel et al. 2009b; Grant and Grant 2010). Because signal displacement in zones of contact can lead to isolation from closely related, yet geographically isolated populations (Hoskin et al. 2005), RCD has been proposed as a powerful means for diversification (Hoskin and Higgie 2010).

However, despite the taxonomic breadth of examples, there are relatively few clear cases of acoustic RCD in nature, and the underlying processes are not generally understood for several reasons. Whereas RCD has been traditionally viewed as a consequence of selection against maladaptive hybridization or misdirected territorial aggression, recent work suggests that RCD could result from a variety of species interactions, including those between distantly related taxa (Hoskin and Higgie 2010). Moreover, convergence rather than displacement of signals in sympatry can be adaptive (Tobias and Seddon 2009).

Because reinforcement implies an inherent cost of hybridization, this mechanism of RCD will generally occur later in the speciation continuum (Figure 2.2, dotted red line). On the other hand, many other proposed mechanisms of RCD, involving competition or predator-prey interactions do not require any genetic incompatibilities between acoustically displaced populations, as divergence occurs as an indirect result of heterospecific interactions. Thus, these mechanisms of RCD might show early or inconsistent patterns of divergence among population pairs (Figure 2.2, dashed green and solid grey line, respectively). Identifying conditions favoring divergence versus convergence, assessing the prevalence of these conditions, and collecting acoustic data for populations differing in genetic relatedness will help clarify the mechanisms and timescale over which acoustic divergence facilitates speciation.

2.9 The role of learning on acoustic divergence

In contrast to genetic mutations, copying errors or novel variants in culturally acquired signals can be transmitted both within and among generations between unrelated individuals (Danchin et al. 2004), and often have higher heritabilities than genetic traits (Danchin et al. 2004). Accordingly, vocal learning has been suggested to accelerate the process of acoustic divergence (Lachlan and Servedio 2004). The fact that oscine songbirds, in which vocal learning occurs, are a very speciose clade has been indicated as evidence that the learning process can facilitate divergence (Lachlan and Servedio 2004) (but see (Baptista and Trail 1992)). However, recent work in diverse suboscine radiations that lack learning suggest that this argument is

weak (Seddon 2005; Seddon and Tobias 2007; Seddon et al. 2008; Tobias et al. 2010), and evidence for a role of learning in accelerating speciation is scarce. Perhaps the only evidence comes from the *Vidua* indigobirds, which are brood parasites of several African estrildid bird species. Within *Vidua,* male song and female preferences are learned through sexual imprinting on host song, resulting in host-specific races of these brood parasites which are reproductively isolated unless host specificity is imperfect (Balakrishnan et al. 2009).

The development of dialects can lead to assortative mating within dialect boundaries, as local song can indicate overall condition or degree of local adaptation (Podos and Warren 2007; Stewart and MacDougall-Shackleton 2008). However, learning could impede speciation (Seddon and Tobias 2007; Olofsson et al. 2011) if post-dispersal learning occurs (Podos and Warren 2007; Seddon and Tobias 2007; Olofsson et al. 2011), as this would remove the link between acoustic signal and local adaptation and facilitate hybridization between incipient species on secondary contact (Olofsson et al. 2011).

Our understanding of the role of learning in speciation is partially limited by an incomplete knowledge of which taxa have culturally acquired acoustic signals. Vocal learning occurs in humans and other mammalian lineages, in addition to three orders of birds (Beecher and Brenowitz 2005). Within these taxa, learning provides a rapid means for the accumulation and transfer of mutations within populations; however, we know little about the timing of learning in relation to dispersal, or variation in the strength of preference for local signals. These factors will both affect

the likelihood of forming stable dialects necessary to initiate reproductive isolation. Moreover, for those species which do form dialects, strong selection for improved learning programs should result in genetic assimilation of species-specific signals (Irwin 2012). However, there are no clear examples of this in the literature (Price 2008) and the role of genetic assimilation in population divergence remains untested. Future work should also aim to assess how novel constraints on "acoustic windows" (Luther and Derryberry 2012), as well as neutral and adaptive processes (Byers et al. 2010a), shape learned signals over time, and how each of these processes contributes to population divergence.

2.10 Suggestions for future research

Integrate studies of adaptive processes of acoustic divergence.

We advocate that the role of sexual selection in signal divergence be explored within systems where research has generally focused on ecological selection, and vice versa. In addition, the heritability of acoustic traits, and therefore their availability to selection, is not known for most systems, due to the inherent difficulty of sampling parent and offspring signals across years in the wild (but see (Grant and Grant 2010)). Thus, future work should aim to bridge the gap from lab to field in order to benefit from systems in which much is already known about the genetic architecture of signal evolution and mate preferences. Such approaches (recently applied to zebra finches (Woodgate et al. 2012) and crickets (Rodríguez-Muñoz et al. 2010)) provide an unprecedented opportunity to ground-truth assumptions and connect experimental and genetic data to biological reality in the wild.

Incorporate receiver perception.

Most speciation studies have focused on signal divergence. However, it is becoming increasingly apparent that receivers can modify their behavior based on subtle variation in signal structure (Tobias and Seddon 2009; Pennetier et al. 2010; Seddon and Tobias 2010; Akre et al. 2011; Amézquita et al. 2011; Grace and Shaw 2012). Meanwhile, overt signal divergence might not be meaningful to receivers (Gee 2005). Thus, future studies should assess behavioral responses to observed signal variation in order to determine its relevance to population divergence.

Broaden consideration of sender-receiver dynamics & social selection.

Very few studies consider the evolutionary significance of female traits or intrasexual and heterospecific sender-receiver dynamics. However, new studies increasingly show the importance of female signals in reproductive isolation, whether as signals used by males in mate-choice or by females in resource defense (Tobias et al. 2011; Tobias et al. 2012). Moreover, recent evidence suggests that heterospecific competition can occasionally drive signal convergence in both sexes through social selection (West-Eberhard 1983) for defense of resources (Tobias and Seddon 2009), especially in tropical birds with year-round territoriality, where acoustic signals mediate both social and mating contexts throughout the annual cycle (Tobias et al. 2011). As such, we suggest a broader scope for defining sender-receiver acoustic communication to include intrasexual communication, female signalers, and the influence of heterospecifics, in both reproductive and non-reproductive contexts.

2.11 Conclusions

Here we synthesize research on the role of adaptive and neutral processes in driving acoustic divergence and speciation. Because research has typically not considered each of these processes within the same study system, we summarize a set of testable predictions to determine the relative importance of each to acoustic divergence (Table 2). Additionally, sensory drive has been a focus of a number of studies on acoustic divergence, yet we argue that this framework does not itself provide an explanation for the underlying mechanisms of acoustic divergence. The acoustic window concept offers a way to incorporate sensory drive considerations of habitat- and sensory-based constraints on standing variation and evolutionary opportunity to investigations of the mechanisms shaping acoustic variation within and between populations. Thus, an analysis of limits to acoustic signal divergence is complementary to, not competing with, models of adaptive or neutral acoustic divergence. We suggest that applying this conceptual framework broadly to the study of acoustic divergence will help to better understand the determinants of divergence and the timescale over which it contributes to speciation. Further, we suggest that this framework is easily adaptable to other behavioral and morphological features which mediate mate selection and conspecific recognition within and among closely related populations.

CHAPTER 3

MULTIMODAL COMMUNICATION IN THE NORTH AMERICAN BARN SWALLOW: INFLUENCES OF INTRA- AND INTERSEXUAL SELECTION ON SIGNAL EVOLUTION [2]

3.1 Abstract

Complex signals, involving multiple components within and across modalities (e.g. visual or acoustic), are ubiquitous in animal communication. Numerous studies show benefits of complex signals in reducing search costs and maximizing signal efficacy; however, much of this work has manipulated the presence or absence of particular signaling modalities, which may miss important interactions of multiple components within modalities. Additionally, researchers have often focused on the role of female preferences in driving complexity, ignoring the contribution of intrasexual competition. Here, we utilize the North American barn swallow (*Hirundo rustica erythrogaster*) to demonstrate a novel approach for understanding the evolution of complex communication systems, in the context of male and female receivers. We integrate model testing with correlation-based phenotype networks to assess whether the *principle of robust over-design* predicts the topology of phenotype networks involved in intra- and intersexual signaling. We find that different signaling traits likely evolved via female choice versus male-male competition, with little signal overlap across contexts. Robust over-design explains the evolution of signal redundancy via intersexual, but not intrasexual selection. We suggest that applying our approach broadly across taxa and sender-receiver contexts will afford researchers new insight into the selective pressures and constraints affecting the evolution of communication.

---

[2] This work was undertaken in collaboration with M. B. Joseph, J. K. Hubbard, and R. J. Safran. This manuscript is currently in review.

3.2 Introduction

Sexual selection has produced a seemingly boundless variety of traits utilized for assessing potential mates and competitors. Striking elaborations in visual, acoustic, olfactory, and chemical cues have intrigued biologists since the inception of the field, leading to thousands of studies on the function of these signals in communication. Given that animals commonly signal in multiple modalities (e.g. visual and acoustic channels) simultaneously (Candolin 2003; Partan 2004; Hebets and Papaj 2005; Partan and Marler 2005), tremendous effort has also gone into understanding when and why multimodal signals should be selectively favored over simple signals (Møller and Pomiankowski 1993; Johnstone 1995; Candolin 2003; Hebets and Papaj 2005; Partan and Marler 2005; Hebets 2011; Higham and Hebets 2013). Multiple redundant signals could be favored if they act as 'backups' to ensure signal receipt. Alternatively, nonredundancy of multiple signals would be favored if 'multiple messages' are more informative, decreasing search costs and reinforcing honesty (Møller and Pomiankowski 1993; Johnstone 1996). Recent game theoretical work also suggests that signal complexity (involving multicomponent signals in one modality or multimodal signals) should generally be favored in the presence of noise, multiple audiences, limited signal bandwidth, and variation in signal honesty (Wilson et al. 2013). However, to date, no well-supported theoretical basis exists for predicting when signal complexity should be favored within versus across modalities, and how this should relate to signal redundancy. Here, we propose a phenotype network-based approach as a powerful tool for understanding biologically

relevant signal complexity. This method aids in visualizing complex data, which has been identified as a major challenge for researchers (Partan 2013), as well as formulating testable predictions, and understanding how sender-receiver dynamics and life history constraints interact in the evolution of animal communication systems. To apply this framework, we utilized a multi-year observational dataset on the North American barn swallow (*Hirundo rustica erythrogaster*). In light of various studies demonstrating that different receivers attend to different aspects of multicomponent traits (Andersson et al. 2002; Collins 2004; Dalziell and Cockburn 2008; Collins et al. 2009), we furthermore assess the roles of competition and female choice in the evolution of signal complexity within and across signaling modalities.

A network-based conceptual framework for understanding signal evolution:

In recent decades, a focus on the effects of 'cue-isolation' across modalities has allowed researchers to disentangle many facets of how signals combine to influence receivers—e.g. dominance, additive or emergence effects; (Partan and Marler 1999; Hebets and Papaj 2005; Partan and Marler 2005; Hebets et al. 2013). However, many organisms produce and use multicomponent signals within a modality, as well as multiple signals across modalities in social interactions (Candolin 2003). A charismatic example is the elaborate courtship behavior of the peacock spider, involving multicomponent visual and acoustic displays (Girard et al. 2011). Experiments manipulating the presence or absence of particular modalities may thus overlook the importance of complex signal interactions among components within modalities (Hebets 2011; Smith and Evans 2013). Therefore, approaches that

identify signaling trait clusters (within and across modalities) important to receivers in different contexts can reveal biologically relevant complexity in communication systems.

Application of network theory affords one possible solution to this problem. A recent theoretical study demonstrated that optimal biological signal design should stem from distributing informational units across multiple weakly correlated modalities, comprised of tightly intercorrelated traits (Ay et al. 2007). This arrangement allows for 'robustness' to signal degradation (redundant 'backup signals') within modalities, as well as independent information ('multiple messages') across modalities. This balance of redundancy and canalization, first highlighted in gene evolution, has been termed the *principle of robust over-design*, and is thought to be a general rule in biological organization (Krakauer and Plotkin 2005). However, this concept has never been directly tested in signal evolution and offers useful baseline predictions for the clustering of signaling traits under selection regimes favoring efficient information transfer. Figure 1 shows a schematic of potential trait correlations within and across modalities, with figure 1D showing the pattern expected from robust over-design.

Although robust over-design provides clear predictions for patterns of the clustering of signaling traits, it is not clear whether this pattern should apply to signals which mediate mate choice, competition, or both. For instance, in superb fairy wrens (*Malurus cyaneus*), one song type is directed at competitors, while another is involved in female choice (Dalziell and Cockburn 2008). The inherent

differences in sender-receiver dynamics between males and females may favor different patterns of signal clustering and redundancy. In this example, we expect females to select song and plumage traits which provide reinforcing information about various aspects of male quality (such as condition or parasite load), while competing males should select for traits conveying information on fighting ability or aggressive motivation. Moreover, because males may experience high costs of close approach to competitors, signals mediating intrasexual selection may be constrained to long-distance signals.

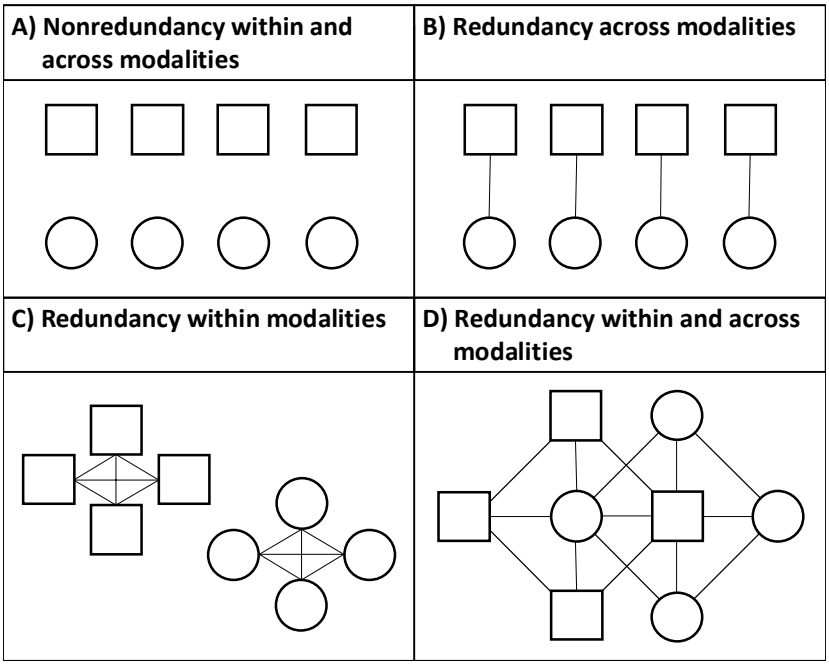

Figure 3.1. A conceptual diagram showing different trait correlations expected for different patterns of signal redundancy within and across modalities. Line connections signify correlations between traits. Nodes represent four different traits in two different modalities (e.g. squares represent morphological features, while circles represent song components.) A) All traits are uncorrelated, potentially signaling independent information, but lack robustness entirely; that is, occlusion of any trait results in information loss. B) Different song and morphological characteristics convey similar information, but there is no correlation among traits within modalities. C) Different morphological and song traits convey similar information, but information conveyed by song and morphology are independent. From both signal design and biological perspectives (Ay et al. 2007), D) illustrates an ideal signaling arrangement. In this case, traits within a modality reinforce each other through a high degree of intercorrelation, while limited correlations between modalities allow for a degree of signal redundancy (and, thus robustness). This is the scenario predicted by robust over-design.

In this study we utilize the baseline predictions of robust over-design as a tool for understanding the complex communication system of the barn swallow. Although the subject of hundreds of sexual selection studies, most work on barn swallows has focused on the role of female choice in signal evolution (in particular, tail streamers within European populations) (Møller 1994, E. Scordato, unpublished manuscript). Here, we consider the functions of and interrelationships between two known targets of sexual selection (tail streamers and ventral color), as well as various aspects of multicomponent song. Our primary aim is to reconcile simplified theoretical models for classifying traits by redundancy or efficiency (Guilford and Dawkins 1991; Guilford and Dawkins 1993; Rowe 1999; Hebets and Papaj 2005; Partan and Marler 2005) with higher-order signal interactions occurring in wild populations. We do this by 1) identifying trait clusters using principle components analysis, 2) performing model selection to determine which trait clusters are implicated in female choice versus male-male competition, 3) developing a phenotype network based on trait intercorrelations to represent the potential for signal redundancy, and 4) integrating the results of model selection with the phenotype network to assess whether robust over-design explains patterns of trait evolution in intra- and intersexual selective contexts.

3.3 Methods

Study System

The barn swallow, *Hirundo rustica*, is a Holarctic-distributed migratory oscine songbird, comprising six described subspecies. Classic research within the European

subspecies *H. r. rustica* has shown that the length of tail streamers (the outermost tail feathers) is under strong sexual selection (Møller 1988). However, research in other barn swallow subspecies now demonstrates great variability in preferences for different feather ornaments (Safran et al. 2005; Hasegawa et al. 2010; Vortman et al. 2011). In particular, within the North American subspecies, *H. r. erythrogaster*, correlational and experimental datasets from New York, Virginia, and Colorado demonstrate that females select for dark ventral plumage, with no preference for tail streamer length (Safran and McGraw 2004; Safran et al. 2005; Neuman et al. 2007; Eikenaar et al. 2011). However, two studies from Ontario, Canada showed that males with longer streamers bred earlier and had a higher proportion of extra-pair offspring in other nests (Smith and Montgomerie 1991; Kleven et al. 2006). Yet, males with experimentally elongated streamers were shown to lose paternity while males with shortened streamers did not (Smith et al. 1991), a result recently replicated in Colorado (Safran, et al. unpublished manuscript). Kleven et al (2005) suggested that conflicting results among populations may result from apparent selection for tail streamer elongation stemming from age-related effects, as tail streamers are known to increase with age (Møller 1994). Thus, within North American populations of *H. r. erythrogaster*, there is strong evidence for selection for dark plumage, while preferences for tail streamer length are currently unclear.

Although nearly all morphological studies of barn swallow sexual selection have focused on female choice, barn swallow song studies demonstrate the clear importance of intrasexual competition. In particular, the length of the rattle (the

terminal trill of songs, figure 3.2B) correlated with testosterone concentration and number of active neighboring males in an Italian population (Galeotti et al. 1997). Additionally, other studies of *H. r. rustica* song in Europe have shown correlations between male immunological variables, song rate (Saino et al. 1997b), peak amplitude of the rattle, and song duration (Garamszegi et al. 2005). Thus, different song components may indicate different aspects of condition; however, which traits are important to females and males remains untested.

Field Methods:

Barn swallows used in this project were part of a long term study conducted between 2008-2012 in Boulder County, Colorado, USA (Latitude 40° 29' 360" N, Longitude 105° 169' 390" W). Ten study sites were used, and each had between 3 and 43 nesting pairs. Each year, barn swallows were captured at the start of breeding using mist nets, banded with USGS metal bands, given a unique combination of a color band and nontoxic permanent ink pen applied to white spots on rectrices. Morphological measures were taken, including right wing length, mass, and the length of streamers (the outermost tail feathers). In addition, contour feather samples were taken from four areas along a ventral transect for later color analysis. During the entire breeding season, we matched banded individuals to nests and monitored reproductive success for all active nests at study sites. Blood samples were taken from adults upon capture and from nestlings on day 12 post-hatching for paternity analyses. All methods described herein were approved by the

University of Colorado Institutional Animal Care and Use Committee (Protocols 07-07-SAF-01 and 1004.01).

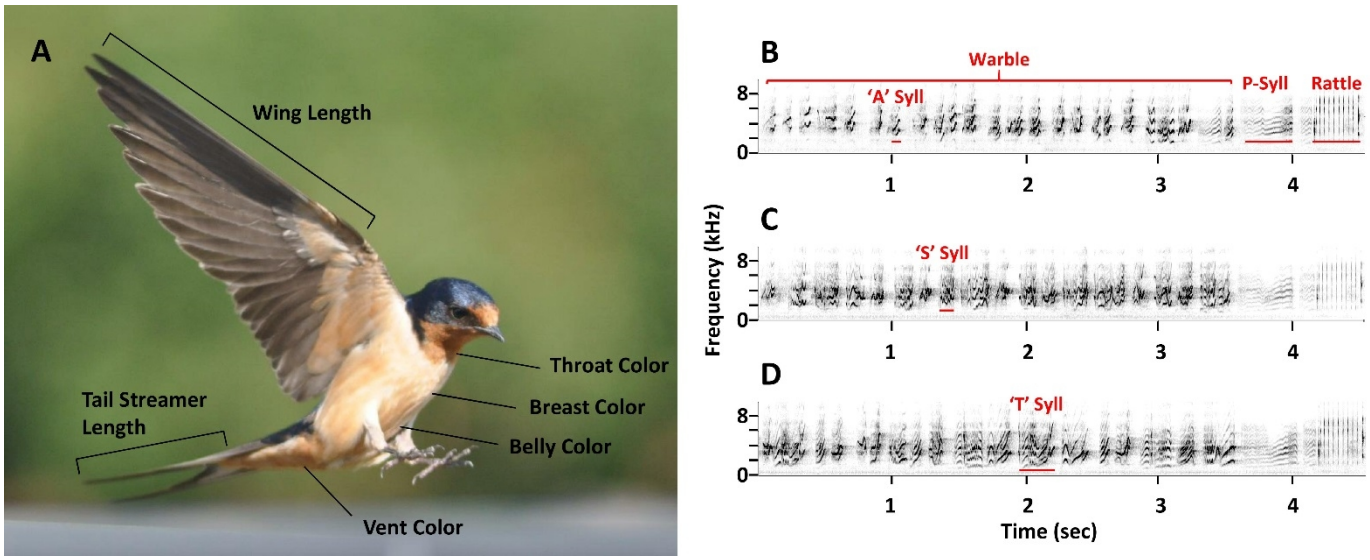

Figure 3.2. Phenotypes measured. Part A) shows locations of morphometric and color measurements on a barn swallow, while B-D are sonograms of three songs produced by the same male. The song in B) is comprised of many simple notes; C) demonstrates intermediate complexity, with many "scratchy," atonal syllables; while D) demonstrates high complexity and tonality.

Color and Song Measurements:

Following Safran et al. (2010), we measured feather color for samples collected from four ventral patches (throat, breast, belly, vent). For each patch, we calculated average brightness, hue, and red chroma. In addition, between three and 20 songs (mean ± SE, 10.67 ± 0.6733) were recorded for 66 males between 5am and 1pm over the following dates: May 15-July 19, 2009; May 6-May 31, 2011; and May 1-August 21, 2012. Only complete songs, comprised of a warbling series of syllables not separated by more than 0.2 s and terminating in a harsh trill (the rattle), were considered for this analysis (figure 3.2B). Song variables (table 3.1 and figure 3.2) were chosen to measure a large portion of song variation, including frequency and

temporal parameters, repertoire size, and composition. In order to determine an appropriate minimum number of songs to estimate song traits, we performed a rarefaction analysis, whereby we assessed the change in a given song parameter estimate by incorporating an additional song sample. For most traits, two songs were sufficient for relatively accurate parameter estimates (appendix 8A3.2). However, consistent with (Garamszegi et al. 2005), we found that 5 songs represented an adequate compromise between underestimating repertoire size and reducing sample size. Also, similar to Saino et al. (1997b), we did not find any effect of recording date or recording date relative to clutch initiation on the song parameters measured here (unpublished data). We therefore utilized the recording date with the most complete songs for data extraction if males were sampled multiply. After setting the 5 song threshold for estimating all song parameters, our dataset consisted of 49 males with complete morphological, color, and song data. Of these, 44 also had paired social reproductive data, and 26 had genetic paternity data.

Table 3.1. Measured phenotypic traits

Category	Subcat	Trait	Description	Mean (SE)
Song Traits				
	Time Domain			
		WL	Warble length—distance between peak of first syllable and peak of last syllable before P (sec)	3.08 (0.11)
		PL	P-syllable length—distance from beginning to end of P-syllable (sec)	0.31 (0.01)
		RL	Rattle length—distance between the first and last pulses in the terminal trill (sec)	0.33 (0.01)
		RTmp	Rattle tempo—number of rattle pulses/ rattle length (Hz)	31.23 (0.27)
		WTmp	Warble tempo—number of syllables before P/ warble length (Hz)	4.56 (0.06)
	Frequency Domain			
		PF W	Peak frequency of the warble—frequency at the maximum amplitude in the warble (Hz)	3960.28 (56.24)
		PF P	Peak frequency of the P-syllable—frequency at the maximum amplitude in the P-syllable (Hz)	4291.46 (75.81)
		PF R	Peak frequency of the rattle—frequency at the maximum amplitude in the rattle (Hz)	5416.32 (100.38)
		BW R	Frequency bandwidth of the rattle—song frequency bandwidth above a -10dB threshold, relative to peak frequency, averaged across the rattle (Hz)	2684.75 (105.58)
		WE W	Wiener entropy of the warble—ratio of the geometric mean to the arithmetic mean of the warble spectrum (0=pure tone; 1=random noise)	0.63 (0.01)
	Repertoire			
		%A	% A-syllables—(a measure of low song complexity) number of A-syllables/ total number of syllables; these syllables are simple, resembling contact calls	29.36% (0.01)
		%S	% S-syllables—(a measure of intermediate song complexity) number of S-syllables/ total number of syllables; these syllables are “scratchy” and atonal	9.04% (0.01)

	%T	% T-syllables—(a measure of high song complexity) number of T-syllables/ total number of syllables; these syllables are complex, highly frequency modulated, and tonal	3.45% (0.003)
	Rep	Repertoire size—cumulative number of unique syllables sampled for a given male	27.53 (0.67)
Morphological Traits			
	RWL	Right wing length (mm)	118.48 (0.38)
	TS	Tail streamer length—maximum length of the outermost tail feathers (mm)	91.31 (1.03)
Color Traits*			
	TBri	Average Brightness—the average percent reflectance between 300 and 700 nm; lower values darker	T: 17.85 (0.88)
	RBri		R: 28.99 (0.98)
	BBri		B: 28.41 (1.06)
	VBri		V: 21.44 (0.68)
	THue	Hue—the wavelength at maximum slope; low values pale/yellowish, high values dark/reddish (nm)	T: 654.28 (3.48)
	RHue		R: 631.79 (3.34)
	BHue		B: 627.09 (4.34)
	VHue		V: 646.62 (3.62)
	TChr	Chroma—the proportion of light reflected in the red color range (600-700 nm); higher values darker	T: 0.5052 (0.01)
	RChr		R: 0.4546 (0.01)
	BChr		B: 0.4486 (0.01)
	VChr		V: 0.4976 (0.01)

*Each color axis measured for T=Throat, R=Breast, B=Belly, and V=Vent

Table 3.2 Variables used in model selection

Predictor variables (rotated principle components of phenotype)	
Variable:	**Higher Value Indicates:**
[Breast Paleness]	Lighter breast and belly
[Song Performance]	Higher pitch, less tonal, narrower frequency bandwidth, with more intermediately complex ('S') syllables
[Rattle Performance]	Faster warble tempo, with shorter, faster rattles
[Feather Length]	Longer wings and tail streamers
[Complexity/Vent Paleness]	More complex ('T') syllables and lighter vent
[Breast Hue]	Redder breast and belly
[Throat Darkness]	Darker, redder throat
[Monotony]	Longer songs, comprised of many simple ('A') syllables
[Repertoire]	Larger cumulative number of syllables

Response variables (metrics of fitness and competition)	
Variable	**Description**
BREEDING ONSET	Difference between Julian date of day first egg was laid by focal males' mate and population average for that year; Gaussian
FLEDGING	Number of fledged young across all broods in focal males' nest(s); Poisson-distributed
PATERNITY	Proportion of genetic offspring in a focal male's nest; Binomial—in logistic regression, number of within-pair young=wins, number of extra-pair young=losses
DISTANCE	Distance to the nearest nest with a fertile female at the site and day a male was recorded; females were considered fertile if the day of recording was within the range of 7 days before clutch initiation and the day prior to clutch completion; Gaussian after log transformation

Data Reduction:

In order to minimize the dimensionality of our data, and explore phenotype associations, we began by only extracting song components which we hypothesized *a priori* to represent important aspects of male quality or motivation, informed by previous barn swallow song studies (Galeotti et al. 1997; Saino et al. 1997b; Garamszegi et al. 2006a). We then chose the component we deemed most biologically relevant in the case that two song variables had a correlation greater than 0.8. Although chroma and average brightness show similarly high correlations, we left both color metrics in our dataset, as they were recently shown to reflect different levels of environmental versus genetic control and may have independent signaling value (Hubbard, JK, et al, unpublished manuscript). Wing length and tail streamer length were also included, as they both increase with age (Møller et al. 1994, Wildrick, R, et al., unpublished manuscript), though there is conflicting evidence on preferences for streamer length in mate choice within the North American subspecies (Smith and Montgomerie 1991; Safran and McGraw 2004; Kleven et al. 2005; Neuman et al. 2007; Eikenaar et al. 2011; Safran, et al., unpublished manuscript). We next performed principal components analysis on the remaining 28 variables (table 3.1), extracting 9 components with eigenvalues over one. These components were then rotated using the varimax method to maximize differences between orthogonal vectors and facilitate interpretation of these latent phenotypic axes. Rotated components were renamed according to trait loadings. Loadings and interpretations of their biological significance are shown in table 3.2.

Paternity Analyses:

As extra-pair copulation is relatively common in barn swallows (Saino et al. 1997c), we assigned paternity to offspring in each focal male's nest using six polymorphic microsatellite markers. We analyzed allele frequencies and performed paternity exclusions using CERVUS 3.0 (Kalinowski et al. 2007). None of the six loci differed from Hardy-Weinberg equilibrium. The probability of correctly excluding a focal male as the genetic father was 0.9891, and given a known mother was 0.9991. Overall rates of extra-pair paternity in nests were 24.0%, comparable to rates found in other barn swallow populations (range: 17.8-34%) (Møller et al. 2006). Additional methods for our measures of color, song, and paternity are available in appendix 8A3.1.

Response variables:

As shown in table 2, we utilized three measures of seasonal reproductive success for each male: 1) BREEDING ONSET, the day the first egg was laid by the social mate; 2) FLEDGING, the number of offspring fledged from the social nest across all broods in the breeding season; and 3) PATERNITY, the proportion of genetically determined within-pair to extra-pair offspring sampled within a male's nest on day 12 post-hatching. These metrics should capture different aspects of realized reproductive performance, and may not exhibit strong intercorrelations. For example, BREEDING ONSET may improve with experience from previous seasons, while increased FLEDGING may result from higher paternal care to ensure fledging success. PATERNITY, on the other hand, represents our best measure of

female choice, as females have been shown to dynamically allocate paternity as a function of changes in phenotype (Safran et al. 2005).

For a measure of intrasexual competition, we used DISTANCE: the (log transformed) linear distance from a focal male's nest to the nearest active nest with a fertile female (and her mate) at the site and day of song recording. We excluded males whose nearest neighbor was more than 12m away from competition analysis, as this was a natural break point in the bimodal distribution of neighbor distances, similar to that observed in (Taff et al. 2013). Previous work has shown that barn swallows maximize distance between each other (Brown 1996), showing a preference for nests hidden from neighbors (Fujita and Higuchi 2006). Moreover, within the European subspecies, males with more active neighbors had higher testosterone levels and longer rattles. These results suggest that DISTANCE is in fact a good metric of intrasexual competition. For clarity, we denote individual traits by short abbreviations (table 3.1); rotated components are contained in brackets (table 3.2); and response variables are in all-caps (table 3.2).

Statistical Approach:

We used an information-theoretic approach to determine which signal axes best explained variation in fitness metrics and competitive environment (Burnham and Anderson 2002; Burnham et al. 2010), as it offers greater power for ranking alternative models and avoids the problem of multiple testing associated with traditional step-wise model selection (Anderson et al. 2000; Whittingham et al. 2006; Burnham et al. 2010; Garamszegi 2010; Symonds and Moussalli 2010). For

each response variable, we specified a global model including all 9 phenotypic factors, with site nested in year as random effects. In the case of BREEDING ONSET, we did not include year as a random effect because clutch initiation was standardized by the population average for each year, such that we would not expect additional among-year variation. The candidate model set for each analysis included every combination of fixed effects, including a minimal model containing only the random effects and a global intercept term, for a total of 512 models for each response variable. This approach is necessary because we had no *a priori* expectations about which combination of traits explained each response (Grueber et al. 2011), and further justified in that each covariate was identified as a biologically relevant phenotypic character reflecting different aspects of male quality. We used model averaging to calculate effect estimates and 95% confidence intervals from models within 2 ΔAIC_c of the best model. Although some authors have suggested this threshold may be too stringent, ignoring important factors found in lower ranking models (Burnham et al. 2010; Grueber et al. 2011; Richards et al. 2011), models greater than 2 ΔAIC_c had model weights below 0.02, indicating very poor fit to the data. General and generalized linear mixed models (LMM and GLMM, respectively) were specified using the 'lme4' package (Bates et al. 2014) and model averaging was conducted using the 'MuMIn' package (Barton 2014) implemented in R v3.0.2 (R Core Team 2014). Models were specified as follows: BREEDING ONSET was a normal LMM, FLEDGING was a Poisson GLMM, PATERNITY was a

binomial GLMM with the number of trials equal to the number of fledglings in a nest, and DISTANCE was a log-normal LMM.

Statistical Inference:

We report model-averaged parameter estimates and 95% confidence intervals for all factors included in our top model set, as this demonstrates the magnitude and precision of each effect. Because we have adopted an information-theoretic approach for inference, we emphasize that these should not be interpreted through a null hypothesis-testing lens (Lukacs et al. 2007; Nakagawa and Cuthill 2007; Burnham et al. 2010). We therefore evaluate models based on AIC_c weights which express the support for each model included in the top model set (Lukacs et al. 2007; Burnham et al. 2010). To further assess model fit, we also calculated marginal and conditional R^2 values (R_m^2 and R_c^2), which represent the variance explained by the fixed effects and both the fixed and random effects, respectively, for each well-supported model (Nakagawa and Schielzeth 2013). Therefore, factors retained in a top model set were the most important for predicting a given response variable, with the index of variable importance (hereafter *importance*, the sum of AIC_c weights of the models that included a factor) acting as a quantitative measure (Burnham and Anderson 2002).

3.4 Results

3.4.1 Objective 1: Identify relationships between variables

Phenotypic traits

Our principle components analysis on 28 morphological, song, and color traits produced 9 factors with eigenvalues over 1, explaining 75% of the cumulative variance (table 8A3.4). These factors included three color axes ([Breast Paleness], [Breast Hue], and [Throat Darkness]), four song axes ([Song Performance], [Rattle Performance], [Monotony], and [Repertoire]), one morphological axis ([Feather Length]), and one multimodal axis ([Complexity/Vent Paleness]). Biological interpretations of factor values are provided in table 3.2.

Response variables

BREEDING ONSET and FLEDGING were correlated, as early-nesting pairs had more time for multiple broods (Spearman's rho, $\rho = 0.418$, $n = 45$, $p = 0.004$). However, proportion of within-pair to extra-pair young was not correlated with either BREEDING ONSET ($\rho = 0.040$, $n = 26$, $p = 0.846$) or FLEDGING ($\rho = -0.265$, $n = 26$ $p = 0.191$). This suggests that males who bred earlier or were better fathers (i.e. fledged more offspring) were not the same as those preferred by females as genetic mates. DISTANCE did not correlate with any other fitness metric (all $|\rho| < 0.234$, $p > 0.169$).

Table 3.3. Best models within 2 ΔAIC_C of the top model for each response variable

Analysis/Candidate Model	df	logLik	AICc	Δ AICc	w_i	acc w_i	R^2_m	R^2_c	ER
BREEDING ONSET									
Site	3	-176.42	359.43	0.00	0.24	0.24	0.00	0.24	--
[Feather Length] + Site	4	-175.38	359.76	0.33	0.20	0.44	0.04	0.28	1.18
[Breast Hue] + Site	4	-175.44	359.88	0.45	0.19	0.63	0.04	0.34	1.25
[Feather Length] + [Breast Hue] + Site	5	-174.30	360.14	0.71	0.17	0.80	0.07	0.38	1.43
[Rattle Performance] + Site	4	-176.00	360.99	1.56	0.11	0.91	0.01	0.27	2.18
[Throat Darkness] + Site	4	-176.14	361.29	1.86	0.09	1.00	0.01	0.23	2.53
FLEDGING									
[Breast Hue] + [Repertoire] + Site/Year	5	-108.64	228.82	0.00	0.47	0.47	0.15	0.30	--
[Breast Hue] + Site/Year	4	-110.26	229.52	0.70	0.33	0.80	0.09	0.28	1.42
[Repertoire] + Site/Year	4	-110.74	230.48	1.66	0.20	1.00	0.07	0.24	2.29
PATERNITY									
[Breast Paleness] + [Feather Length] + Site/Year	5	-36.38	85.76	0.00	0.20	0.20	0.26	0.28	--
[Feather Length] + Site/Year	4	-38.29	86.48	0.72	0.14	0.34	0.18	0.18	1.44
[Feather Length] + [Monotony] + Site/Year	5	-36.80	86.59	0.83	0.13	0.48	0.24	0.24	1.52
[Breast Paleness] + [Rattle Performance] + [Feather Length] + Site/Year	6	-35.10	86.62	0.86	0.13	0.61	0.28	0.34	1.54
[Rattle Performance] + [Feather Length] + [Monotony] + Site/Year	6	-35.20	86.81	1.06	0.12	0.73	0.28	0.36	1.70
[Rattle Performance] + [Feather Length] + Site/Year	5	-36.92	86.85	1.09	0.12	0.85	0.20	0.24	1.73
[Breast Paleness] + [Feather Length] + [Monotony] + Site/Year	6	-35.62	87.67	1.91	0.08	0.92	0.28	0.29	2.60
[Rattle Performance] + [Feather Length] + [Complexity/Vent Paleness] + Site/Year	6	-35.65	87.71	1.96	0.08	1.00	0.23	0.41	2.66
DISTANCE									
[Repertoire] + Site/Year	5	-35.93	83.81	0.00	0.32	0.32	0.10	0.38	--
[Rattle Performance] + [Repertoire] + Site/Year	6	-34.94	84.67	0.87	0.21	0.52	0.14	0.48	1.54
[Song Performance] + [Repertoire] + Site/Year	6	-35.00	84.79	0.99	0.19	0.72	0.14	0.46	1.64
[Breast Hue] + [Repertoire] + Site/Year	6	-35.21	85.22	1.41	0.16	0.88	0.13	0.42	2.03
[Song Performance] + [Rattle Performance] + [Repertoire] + Site/Year	7	-33.91	85.69	1.89	0.12	1.00	0.17	0.55	2.57

3.4.2 Objective 2: Identifying phenotypic traits involved in mate choice and competition

To assess how different traits function in reproductive versus competitive contexts, we performed model selection for three surrogate fitness metrics and one competition metric. Our results, shown in table 3.3, demonstrate that different traits tended to predict different response variables. Model averaged effects and confidence intervals for the top models are shown in figure 3.3. Numerical values for model averaged effects, confidence intervals, and importance values are shown in table 8A3.5.

BREEDING ONSET

The best model for predicting BREEDING ONSET contained no fixed effects and only the random effect site (table 3.3), indicating that differences in clutch initiations across sites outweighed consistent morphological differences. The top model set (including models within 2 ΔAIC_c units of the best model) contained six models, including four factors: [Feather Length], [Breast Hue], [Rattle Performance], and [Throat Darkness] (*importance* = 0.37, 0.36, 0.11, and 0.09, respectively). This indicates that, generally, darker males with long wings and streamers and faster rattles, with broader frequency bandwidth started breeding earlier (figure 3.3A). However, a maximum *importance* value of 0.37, for [Feather Length], indicates that the best phenotypic predictor of clutch initiation was only included in a small subset of top models, and all phenotypic traits had weak support. For the top models, R_m^2 ranged from 0 to 0.074, and R_c^2 ranged from 0.23 to

0.38. As R_m^2 indicates variation explained by the fixed effects, and R_c^2 reflects variation explained by fixed and random effects, these results suggest that site-to-site variability was more important than individual phenotype effects for BREEDING ONSET.

FLEDGING

The best model for FLEDGING included [Breast Hue] and [Repertoire], with a model weight of 0.47. These were the only two factors retained in the top model set (*importance* = 0.80 and 0.67, respectively). The relatively high best model weight and *importance* values for each factor provide moderate support for these traits in determining apparent reproductive performance. Parameter estimates indicate that males with paler (yellow-shifted) breast and belly color and smaller repertoires, fledged more offspring from their nests (figure 3.3B). However, there was considerable unexplained variation in the number of chicks fledged, with

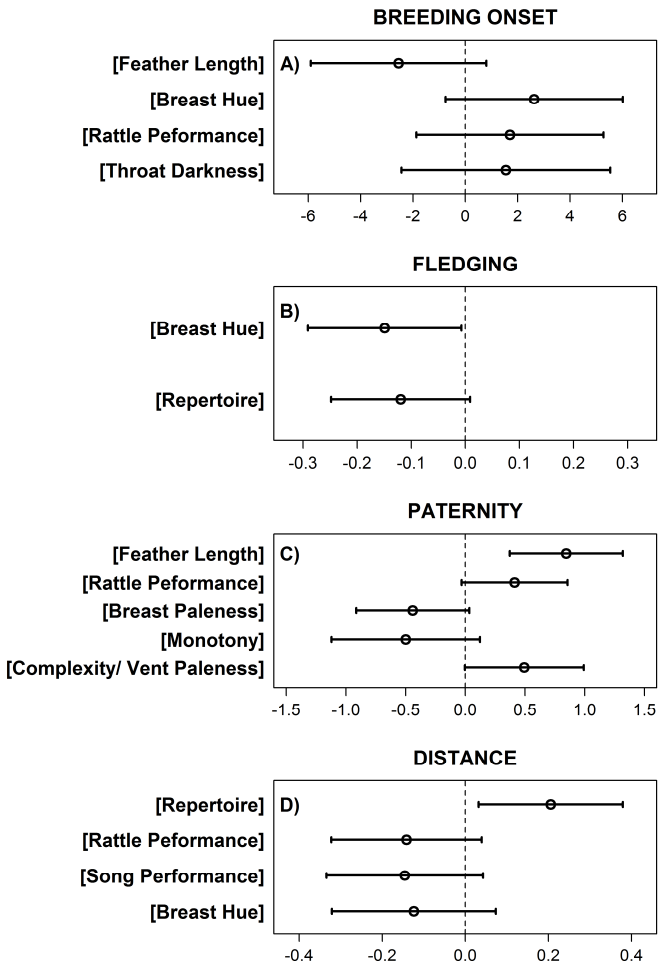

Figure 3.3. Model-averaged slope estimates and confidence intervals for traits ranked in the top 2 ΔAIC_c for each response variable; predictor variables are ordered from greatest to least *importance*, beginning at the top of each graph.

R_m^2values between 0.07 and 0.15 in the top models. There were also major site effects in fledging success, as R_c^2 varied between 0.24 and 0.30.

PATERNITY

The best model for PATERNITY included [Breast Paleness] and [Feather Length], with a model weight of 0.20. The top model set retained 8 models, including 5 factors: [Feather Length], [Rattle Performance], [Breast Paleness], [Monotony], and [Complexity/Vent Paleness] (*importance* = 1.00, 0.44, 0.41, 0.33, 0.08, respectively). [Feather Length] was by far the best predictor of PATERNITY, while there was moderate support for [Breast Paleness] and [Monotony], and minimal support for [Complexity/Vent Paleness]. Thus, males with longer streamers and wings, darker breast and belly, and shorter songs with fewer simple syllables were much less likely to be cuckolded (figure 3.3C). To a lesser degree, males with a greater number of complex ('T') syllables and lighter vent plumage had higher genetic paternity. R_m^2 for the paternity models ranged from 0.18 to 0.28, and R_c^2 ranged from 0.18 to 0.40, suggesting that differences in breeding density across sites did not have major effects on rates of cuckoldry.

DISTANCE

The best model for DISTANCE contained only [Repertoire], with a model weight of 0.32. The top model set contained five models, including four factors: [Repertoire], [Rattle Performance], [Song Performance], and [Breast Hue] (*importance* = 1.0, 0.33, 0.32, 0.16, respectively). All top models contained [Repertoire], and model averaged estimates indicated a strong effect (figure 3.3D), with males with larger

repertoires maintaining a greater distance to nearest neighbor. Overall, males with greater distance to the nearest active neighbor tended to have larger repertoires, paler (yellow-shifted) breast hue, slower, lower pitched, more tonal songs, comprised of fewer 'S-syllables', with slower, longer rattles covering a broader frequency bandwidth. R_m^2 values ranged from 0.10 to 0.17, and R_c^2 from 0.38 to 0.55, indicating considerable among-site differences in nest spacing, consistent with varying levels of breeding density.

3.4.3 Objective 3: Visualizing the phenotype network

To assess whether robust over-design predicts patterns of signal evolution in barn swallow communication, we generated a phenotype network of all 28 measured traits. This network (shown in figure 3.4A) was generated from Spearman's rho correlations $\geq |0.3|$ between all phenotypic traits using the R package 'qgraph' (Epskamp et al. 2012). A minimum correlation of 0.3 was chosen to represent 'medium effect sizes' (Cohen 1988; Nakagawa and Cuthill 2007) and limit assumptions of redundancy between signals. From figure 3A, in which node shapes reflect trait type, it is clear that although most nodes tend to cluster by modality (e.g. strong correlations among color measures), there are also many strong relationships across modalities. For instance, connections between '%T' (the proportion of complex syllables in the warble) and both 'TS' (tail streamer length) and 'VChr' (vent chroma) indicate that males who sang a greater proportion of complex syllables tended to have longer tail streamers and lighter vents. These may be redundant signals, reinforcing some underlying information about male quality.

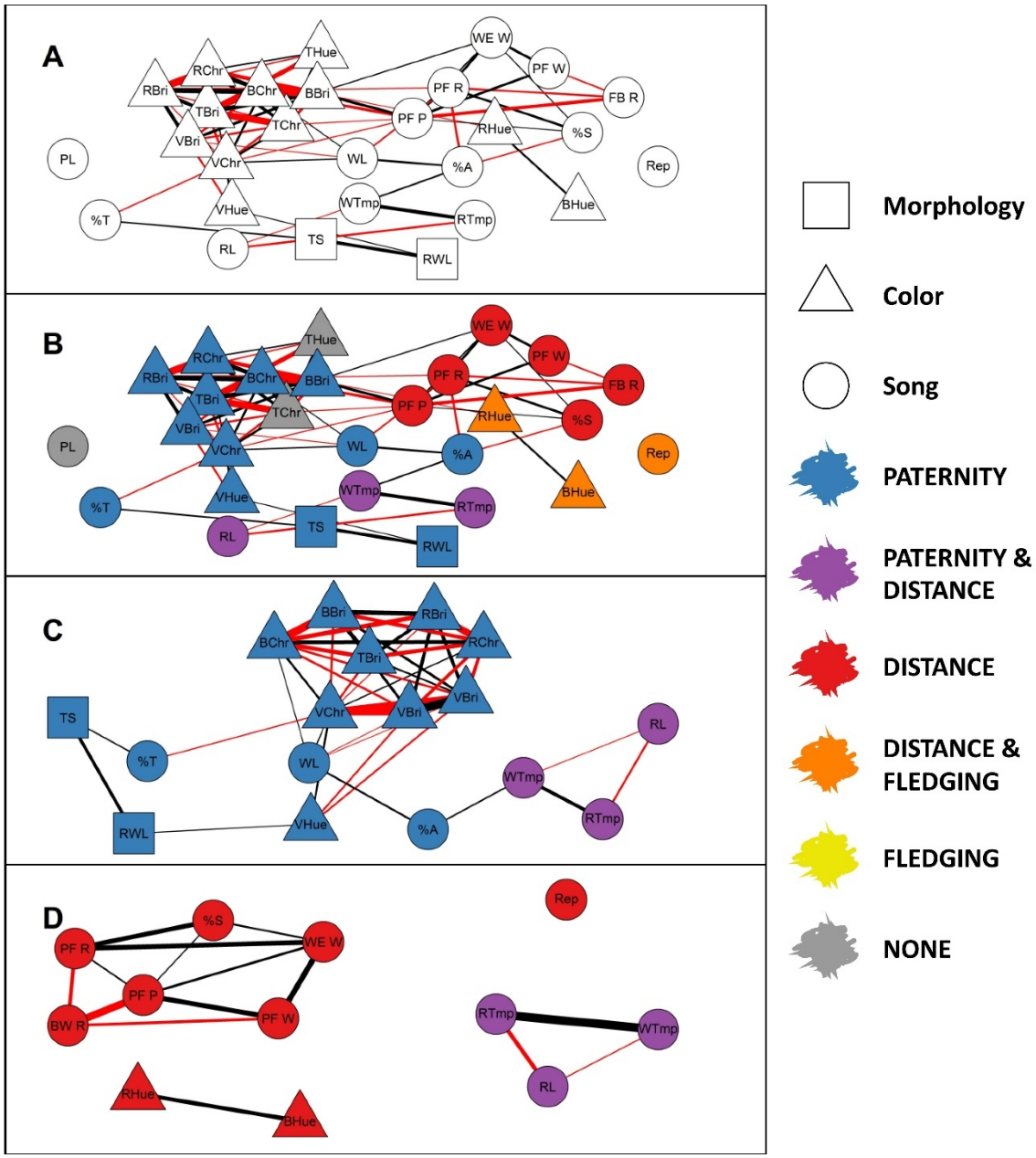

Figure 3.4. The barn swallow phenotype network, showing Spearman's rho correlations between measured traits for N=49 males. The thinnest edge (line connecting two nodes) represents a correlation of |0.3|, and an isolated node signifies no correlation ≥|0.3| with any other trait. Thicker edges connecting nodes represent stronger relationships, with black and red edges indicating positive and negative correlations, respectively. Graph A) demonstrates intercorrelations between all measured traits, with different trait types symbolized by different shapes. Graph B shows the same network, with nodes colored based on loading >|0.5| on factors included in the top models for each response variable. In contrast, graph C) reflects only traits important for determining PATERNITY (i.e. female choice), and D) includes only traits important for DISTANCE (i.e. male-male competition). FLEDGING traits were not included in C and D to emphasize distinctions between signals involved in inter- versus intra-sexual selection. Also, node clustering is determined by a force-directed 'spring' algorithm which minimizes edge crossing (Fruchterman and Reingold 1991). Thus, because A and B include all the traits, and C and D contain different subsets of traits, optimal node arrangements differ among these graphs.

However, as emphasized by (Taylor et al. 2011), signal redundancy cannot truly be inferred without observing responses. That is, because 'TS' loaded on [Feather Length], while '%T' loaded on [Complexity/Vent Paleness], these traits may not function in the same signaling context (i.e. female choice versus intrasexual competition). Therefore, in order to test our hypotheses related to evolution through selection for robust over-design, we integrated our results from model selection with the phenotype network.

3.4.4 Objective 4: Interpreting patterns of signal evolution

In Figure 3.4B, nodes are color-coded according to loading $\geq |0.5|$ on factors included in the top models for FLEDGING, PATERNITY, and DISTANCE. This figure therefore represents the traits likely to be important in each signaling context. BREEDING ONSET was not included, as phenotypes did not explain variation in this response variable. This approach provides simultaneous information on signaling modality, potential for redundancy, and likely function in communication. As shown in figure 3.4B, there is clearly little overlap in the context in which traits are utilized (few orange or purple nodes). In addition, traits important for determining paternity outcomes (blue and purple nodes) are highly clustered, while traits involved in competition (red, purple, and orange nodes) are disjointed and scattered throughout the network. For clarity, PATERNITY and DISTANCE networks are shown separately in figures 3.4C and 3.4D, respectively. Traits implicated as important for determining PATERNITY exhibit several tightly correlated trait clusters, with few connections between (figure 3.4C), as predicted by

robust over-design. In contrast, traits important for determining DISTANCE exhibit four independent clusters (figure 3.4D), consisting of frequency-related song components (top left cluster), repertoire (unconnected circle), performance-related traits (purple nodes), and breast/belly hue (red triangles). This pattern is similar to a combination of figure 3.1A and 3.1C, with many correlations within and no correlations across modalities, but some song components potentially imparting independent information.

No unique set of traits was important for determining fledging success (no yellow nodes in figure 3.4B), as [Breast Hue] and [Repertoire] (comprising the orange nodes) were implicated in both FLEDGING and DISTANCE. It is important to note, however, that the directionality of the [Repertoire] effect was opposite in these two contexts, with males who had low repertoires fledging more offspring and males with high repertoires maintaining a larger distance to the nearest competitor (see figure 3.3B and 3.3D).

3.5 Discussion:

In this study, we quantified associations between multimodal traits, determined their function in intra- versus intersexual selection, and assessed the role of robust over-design in the evolution of signals involved in these two contexts.

Trait correlations:

The fact that our analyses extracted nine orthogonal axes of phenotype which explained a large portion of the variation in our 28 measured traits speaks to the complexity of the barn swallow communication system. That one of the axes

extracted, [Complexity/Vent Paleness], was a multimodal trait also demonstrates that perceived trait clusters may not fall into categories based on modality. Thus, traditional modality-centric approaches to studies of signal complexity could miss important signal interactions if, as here, some components of song are closely intercorrelated, while others are more closely associated with different aspects of phenotype.

Intrasexual selection

In addition to higher repertoires, greater DISTANCE was associated with lower rattle and song performance, and to a lesser degree, yellower breast hue. Although causality is impossible to infer for certain in dynamic song traits, we suggest that dominant males advertise competitive ability by expressing a large number of syllables. Because they are able to maximize a larger distance to their nearest competitor, they are also in a calmer state, producing syllables and rattles at a slower rate. This is consistent with previous findings among *H. r. rustica* in Italy, indicating greater exaggeration of the rattle in the presence of more competitors (Galeotti et al. 1997). Why yellower hue would be associated with more dominant males is unclear.

Intersexual selection

Our results indicate that tail streamer length (together with wing length) was the best predictor of PATERNITY. This result was unexpected, given previous studies showing no relationship between tail streamer and reproductive success in this subspecies (Safran and McGraw 2004; Neuman et al. 2007), and the results of a

recent experiment in our study population wherein males with artificially elongated tail streamers lost paternity in the brood following manipulation (Safran, RJ et al, unpublished manuscript). However, these results somewhat parallel barn swallow studies in Ontario (Smith et al. 1991; Smith and Montgomerie 1991; Kleven et al. 2006). Similar to Kleven et al. (2005), we posit that, as tail streamers and wing length are both known to increase with age in males (Møller 1994, Wildrick, R, et al., unpublished manuscript), this pattern may result from more experienced males mate guarding more. This hypothesis requires further validation.

Signal evolution:

We find support for the principle of robust over-design in the evolution of traits utilized in female choice. This result is intuitive, as females should be interested in minimizing search costs and maximizing information about males. Song, color, and morphological traits should also reflect different developmental aspects of male quality, as these traits differ markedly in their physiological basis and degree of plasticity. Additionally, females may use a few song traits for initial mate assessment and fine differences in visual signals upon closer approach. This was recently shown in house crickets (*Achetus domesticus*), where females preferred playbacks of calling songs of larger males, and also preferred larger muted males presented at close range (Stoffer and Walker 2012). We predict this pattern to exist where selection for effective communication is unconstrained; that is, where fitness or energetic costs of signal assessment are low, and benefits of information transfer are high. Therefore, in barn swallows, where forced copulation of females is rare

(Møller 1985), we infer that strong selection for accurate mate discrimination has resulted in a balance of redundancy within and weak redundancy across signal trait clusters involved in female choice.

In contrast, our results indicate that traits involved in male-male competitive interactions are primarily components of song. Moreover, trait correlation patterns did not conform to our predictions of robust over-design, as we found four potentially non-redundant trait clusters. In barn swallows, male-male competition is very prominent, with frequent, highly physical battles occurring over the breeding season (Møller 1994, MRW personal observation). As males should prefer to minimize costs associated with close approach, they appear to utilize signals that can be perceived with minimal degradation over a distance. Male song represents one such example, as it may be perceived over a large distance, even when the signaler cannot be seen. Work on species recognition in birds indeed suggests sequential assessment of song and visual cues, with song offering the initial assessment from a distance, before closer visual inspection of an intruder (Podos 2010; Uy and Safran 2013). The one color axis shown to be important for male-male competition was [Breast Hue] (i.e. breast and belly hue, figure 3.4D). Previous work in frugivorous birds suggests that measures of chromatic fruit color (such as hue) allow for greater discrimination in variable light conditions (Schaefer 2006; Cazetta et al. 2009). Thus, feather hues of breast and belly (the two most visible plumage patches) likely represent the visual traits which allow males to most reliably assess

potential competitors from a distance within the variable light conditions commonly encountered in barn swallow nesting sites.

In line with Wong and Candolin (2005), we suggest that consideration of sender and receiver life history traits lends greater insight for predicting patterns of signal evolution. Specifically, we predict that costly competitive interactions may sometimes constrain the evolution of signals to modalities which may be perceived and discriminated reliably from the maximum distance. The use of complex chemical and acoustic signaling in the establishment of territories in many predators illustrates the broad generality of this principle. Further, signal nonredundancy should be favored in such cases, as this will allow males to assess different aspects of potential competitors efficiently. For example, in fallow deer (*Dama dama*), formant frequencies honestly reflect size, fundamental frequency changes in relation to dominance (Vannoni and McElligott 2008), and both of these call features may signal age (Briefer et al. 2010), while rate and overall quality of calls likely indicate motivation and condition of rivals (Pitcher et al. 2014). In contrast, where interactions are not costly (e.g. female choice in species with low levels of forced copulation, or ritualized, nonviolent male competition), receivers should select for high signal redundancy within and weak redundancy across modalities, as this will maximize stimulation and memory, reduce search costs, and reinforce honesty in variable environments (Møller and Pomiankowski 1993; Rowe 1999; Candolin 2003; Bro-Jørgensen 2010).

Multiple mating strategies:

Interestingly, we found a negative relationship between FLEDGING and PATERNITY. Although not significant, given our reduced sample size, this result is suggestive of multiple mating strategies. That is, dark males with long tail streamers, complex song, and faster rattles have a high proportion of genetic offspring within their own nests, and likely in the nests of their less attractive competitors (e.g. Kleven et al. 2005). Evidence from studies in Europe, Japan, and the US, however, demonstrate no direct benefits of mating with preferred males (Møller 1994; Kojima et al. 2009; Maguire and Safran 2010). Instead, pale, yellower males with low repertoires may compensate with greater paternal care effort to maximize realized reproductive success. The presence of two reproductive strategies would therefore explain why the only study to consider the effects of color on fertilization success across all nests in a North American population found no difference between dark and drab males (Eikenaar et al. 2011).

Intriguingly, there was also no overlap in the phenotypic traits which predicted FLEDGING and PATERNITY. Instead, FLEDGING traits were related to DISTANCE, with males with small repertoires fledging more offspring, but maintaining a shorter distance to the nearest competitor. Although repertoire size has often been suggested to result from female preference for elaborate songs (Catchpole 1987; Searcy 1992; Catchpole and Slater 2003), recent work indicates weak support for this hypothesis across birds (Byers and Kroodsma 2009; Cardoso and Hu 2011; Price 2013). One possibility is that repertoire size is a byproduct of

selection for song-matching as a means for mediating male-male territorial aggression (Byers and Kroodsma 2009). In our population, it is therefore possible that small song repertoire advertises subordinacy to competing males, resulting in fewer agonistic interactions and allowing for greater attention to paternal care. Further work is necessary to assess this possibility. The fact that females may benefit from pairing with a nurturing, subordinate male, and allocating genetic paternity to desirable extra-pair mates, would also explain a lack of correlation between any of our fitness metrics and competition.

When do males and females select for the same traits?

Our results demonstrated very little overlap in the signals involved in intra- versus intersexual selection. Directional selection by males and females is expected to be reinforcing in systems (like barn swallows) where contests and mating decisions are ongoing throughout the breeding season (Hunt et al. 2009). However, predicting which signaling aspects are reinforced should be a goal for future research. Previous work in black-capped chickadee song, for example, illustrates that eavesdropping females reduced paternity to high-ranking mates after playback simulated loss in a competitive bout (Mennill et al. 2002). Yet, barn swallow song is much more complex than chickadee song, so if females benefit from information about a male's competitive ability, it is unclear which traits should be co-opted for mate choice.

It is telling that the only song traits selected by both males and females in our study were related to a triad involving rattle length, warble tempo, and rattle tempo (purple nodes in figure 3). Trait intercorrelations show that fast warbling males also

had fast rattles, while both these song metrics traded off with rattle length. Similar to performance tradeoffs shown in a number of bird species (Podos 1997; Podos 2001; Ballentine 2004; Wilson et al. 2014), it is not possible to maximize rattle length and tempo at the same time. Given the known importance of the rattle mediating territoriality in European populations (Galeotti et al. 1997), females could gain information on a male's relative quality, conveyed within a mere 0.33s (±0.01 SE). Such high performance signals have been proposed as 'index traits' as they are impossible to fake, and represent an instantaneous assessment of quality (Smith and Harper 1995). Alternatively, these traits could reflect early developmental stress (Nowicki and Searcy 2004; Nowicki and Searcy 2005), as only males with high physical and neurological development should be able to perform well. However, it is also possible that females simply select for those signals which are easiest to assess. A previous study demonstrated stronger female preferences for shorter signals across a broad sampling of insects and anurans (Reinhold 2011). This may be due to the cognitive difficulty of processing longer signals (Akre et al. 2011; Reinhold 2011), but also may result from a biological tendency toward efficient communication through compression (Ferrer-i-Cancho et al. 2013). Regardless of the ultimate cause, our results are consistent with those from several bird species which have been shown to prefer performance-related traits (Vallet et al. 1998; Drăgănoiu et al. 2002; Ballentine 2004). It was also recently suggested that these traits may be more common targets of female selection than is currently appreciated (Byers et al. 2010b). Therefore, future studies may find that

performance traits are commonly targets of reinforcing intra- and intersexual selection.

3.6 Conclusion:

Consistent with a growing number of studies across disparate taxa, our results demonstrate multiple traits, across visual and acoustic modalities, which are involved in female mate choice. However, an entirely different set of traits predicted fledging success, and a negative correlation between these metrics of fitness may indicate the existence of multiple reproductive strategies. Moreover, we found that high performance song traits were the only signals which mediated both female choice and male-male competition. We suggest this may be a common pattern across taxa, as these traits allow for instantaneous assessment of condition or quality by both rivals and potential mates. Furthermore, by assessing the connectivity of trait clusters in phenotype networks, we found strong support for the principle of robust over-design in explaining the evolution of complexity in traits evolved through intersexual selection. In contrast, we found that traits mediating intrasexual competition were constrained to long-distance signals, which has probably led to selection for nonredundancy at the cost of signal robustness. We therefore propose that robust over-design should explain the evolution of communication systems where there is strong selection for efficient information transfer and low cost of assessing multiple signals (such as in female choice across taxa, flower-pollinator interactions, and aposematic signaling). The absence of patterns expected by robust

over-design should direct researchers to important life history traits which may constrain the evolution of complexity.

Future Directions

We hope the methodology utilized here will afford researchers new insight into biologically relevant complexity in animal communication systems and provide fodder for future experiments on signal interactions and relative signal weighting in receivers. Particularly promising are techniques proposed by Smith and Evans (2013) for manipulating traits within the range of natural variation and visualizing response surfaces. These methods will allow for stronger inference about how receivers perceive signal variation and respond in different environments. Another major goal going forward will be to build on a growing literature on the design, signaling context, function, and redundancy of complex signals to build models for predicting patterns of signal evolution across taxa. Such approaches, recently applied to morphological evolution in mosquitofish (Langerhans 2010), offer a promising new synthetic tool. By developing models of signal evolution which consider the principle of robust over-design, as well as selective pressures and constraints imposed by different sender-receiver dynamics, researchers will be able to highlight areas of consensus and high predictability of evolution, and direct research to areas of poor model prediction.

CHAPTER 4

SONG DIVERGENCE ACROSS THE BARN SWALLOW SPECIES COMPLEX [3]

4.1 Abstract

Research across diverse taxa, including mice, birds, frogs, and arthropods provides mixed evidence for a role of stochastic or deterministic processes in driving geographic variation in acoustic signals. Thus, it is unclear whether isolation by distance or isolation by adaptation provides a better general explanation for acoustic divergence across taxa. Similarly, we have no clear predictions for whether geographic overlap of recently diverged populations should lead to increased similarity or dissimilarity of signals. Here, we provide the first description of geographic variation in the barn swallow species complex (*Hirundo rustica*), sampling from five of six described subspecies, spanning much of the species range across the Northern Hemisphere. We note broad syntactic similarity across this range, with greater divergence in the temporal patterning of songs than frequency characteristics across subspecies. Moreover, our results do not support isolation by distance for acoustic divergence. Instead, we suggest that divergent selection pressures better explains signal differentiation among populations. One potential source of selection is for signal convergence in sympatry with competing subspecies, as we noted the songs of *H. r. rustica* became more similar to *H. r. tytleri* in a contact zone in Russia. Collectively, this work adds key information on song divergence for an important model of sexual selection and speciation research, and provides further evidence for the importance of localized selection pressures in driving the evolution of birdsong.

---

[3] This work was conducted in collaboration with S.-F. Chan, H. Karaardıç, P. L. Pap, A. Rubtsov, E. S. C. Scordato, G. Semenov, S.-F. Shen, Y. Vortman, and R. J. Safran

4.2 Introduction

A diverse range of animals use acoustic signals to mediate competition and mate choice within species. Because of the long-range capabilities and rapid evolvability of these traits, acoustic signals have been proposed to be particularly important in the formation of pre-zygotic barriers to reproduction which may initiate speciation (Jones 1997; Wilkins et al. 2013). Birdsong, in particular, has been a major focus of research in population divergence, as song has been linked to immunocompetence (Saino et al. 1997b; Møller et al. 2000; Garamszegi et al. 2005), heterozygosity (Reid et al. 2005b), competitive ability, testosterone levels (Galeotti et al. 1997), and parasite loads (Møller et al. 2000). Moreover, while other vocalizations (such as alarm calls) may be directed at heterospecifics, birdsong is specifically targeted at potential competitors and mates, and is therefore subject to sexual selection pressures (Collins 2004). Because sexual selection is influenced by the ecological context in which signals evolve, such as altitude (Snell-Rood and Badyaev 2008), latitude (Weir and Wheatcroft 2011), or habitat structure (Tobias et al. 2010), song variables may generally reflect degree of local adaptation (Stewart et al. 2009), essential for isolating populations in the early stages of divergence. Therefore, birds represent a major study system for understanding the dynamics of signal evolution, and analyses of within- and among-population variation can be important for illuminating questions related to the evolution of communication systems and the propagation of biodiversity.

One outstanding question in signal evolution is whether stochastic processes influencing signal characteristics over geographic space (isolation by distance) or deterministic processes, such as divergent ecological selection, sexual selection, or character displacement (isolation by adaptation) are more important in explaining geographic variation in acoustic signals (Wilkins et al. 2013). Evidence from mice (Campbell et al. 2010), frogs (Prohl et al. 2006; Amézquita et al. 2009), and birds (Irwin et al. 2008) implicate an important role in genetic and/or cultural drift in the evolution of signals. However, in the last example (the greenish warbler, *Phyloscopus trochiloides*), sexual selection is thought to act in combination with drift to produce patterns of song divergence (Irwin et al. 2008). In contrast, ecological selection is thought to be the primary driver of acoustic divergence in other species of birds (Patten et al. 2004; Ruegg et al. 2006; Kirschel et al. 2009a; Tobias et al. 2010) and at least one species of frogs (Ryan et al. 1992). Sexual selection has been considered most important for driving acoustic signal divergence in bird (Irwin 2000; Price and Lanyon 2004; Seddon et al. 2008), arthropod (Fitzpatrick and Gray 2001; Mendelson and Shaw 2005; Rodríguez et al. 2006; Sullivan-Beckers and Cocroft 2010), and frog (Boul et al. 2007) species. However, several authors have also stressed the importance of character displacement through interspecies interactions (Grether et al. 2009), or as a result of reinforcement to minimize the production of costly hybrids in recently diverged taxa (Hoskin et al. 2005; Kirschel et al. 2009b). In contrast, researchers have sometimes documented convergence of signals in sympatry, owing to the age of the contact zone

or degree of interspecific competition (Haavie et al. 2004; Tobias and Seddon 2009). Thus, we currently have no clear expectations for whether isolation by distance through stochastic processes or isolation by adaptation through deterministic processes should prevail in driving acoustic divergence for a given taxon, or whether divergence or convergence should be adaptive in sympatry upon secondary contact.

The barn swallow has been a fixture of sexual selection research for decades. However, song has only been studied within the European subspecies, and it is not clear to what degree songs have diverged across the enormous Holarctic range of this species. Moreover, because the barn swallow species complex is thought to have radiated out of Africa within the last 100,000 years (Zink et al. 2006; Dor et al. 2010), this is an ideal system for investigating patterns of isolation by distance versus isolation by adaptation in driving signal divergence. This paper aims to: 1) provide the first description of geographic variation in barn swallow song, 2) assess whether geographic variation in song is best explained by isolation by distance or isolation by adaptation, 3) determine which song components best discriminate between subspecies, and 4) assess whether character displacement or convergence occurs within a subspecies contact zone.

4.3 Methods

Study System

The barn swallow, *Hirundo rustica*, is a Holarctic-distributed migratory oscine songbird, comprising six described subspecies (Dor et al. 2010), although eight are sometimes recognized (del Hoyo and Elliott 2014). Classic research within the

European subspecies *H. r. rustica* has shown that the length of tail streamers (the outermost tail feathers) is under strong sexual selection (Møller 1988). However, research in other barn swallow subspecies now demonstrates great variability in preferences for different plumage traits (Safran et al. 2005; Hasegawa et al. 2010; Vortman et al. 2011). In particular, within the North American subspecies, *H. r. erythrogaster*, females select for dark ventral plumage, rather than elongated tail streamers (Safran and McGraw 2004; Safran et al. 2005; Neuman et al. 2007; Eikenaar et al. 2011). However, song characteristics have only been studied within the European subspecies (*H. r. rustica*), and we currently do not know the degree to which song has diverged among closely related populations.

Field Methods:

All described methods were approved by the University of Colorado Institutional Animal Care and Use Committee (Protocols 07-07-SAF-01 and 1004.01).

Song Analyses:

Between three and 20 songs were recorded for 180 male barn swallows over five years. Sampling included five of six described subspecies, recorded at 19 sites within six countries (figure 4.1). Sampling site information is shown in table 4.1. Recordings were taken during the period of high song activity between 5am and 1pm over the following dates: *in the US,* May 15-July 19, 2009; May 6-May 31, 2011; and May 1-August 21, 2012; *in Turkey,* April 20-July 4, 2010; *in Israel,* May 7-9,2010; *in Romania,* June 19-22, 2010; *in Taiwan,* June 3-8, 2011; and *in Russia,* May 21-July 24, 2013. All songs were recorded in 16-bit WAV format, with 48kHz

sampling rate using a Marantz PMD 660 digital recorder, paired with an Audiotechnica AT815B shotgun microphone, a Marantz PMD 660 paired with a Sennheiser MKH 20 and Telinga parabola, or a Marantz PMD 661 paired with a Sennheiser ME62/k6 microphone and Telinga parabola. Banded males were identified by unique combinations of permanent ink marker (Sharpie) colors applied to white dots on rectrices and a color band, while unmarked males were differentiated by physical characteristics and distance between singing territories.

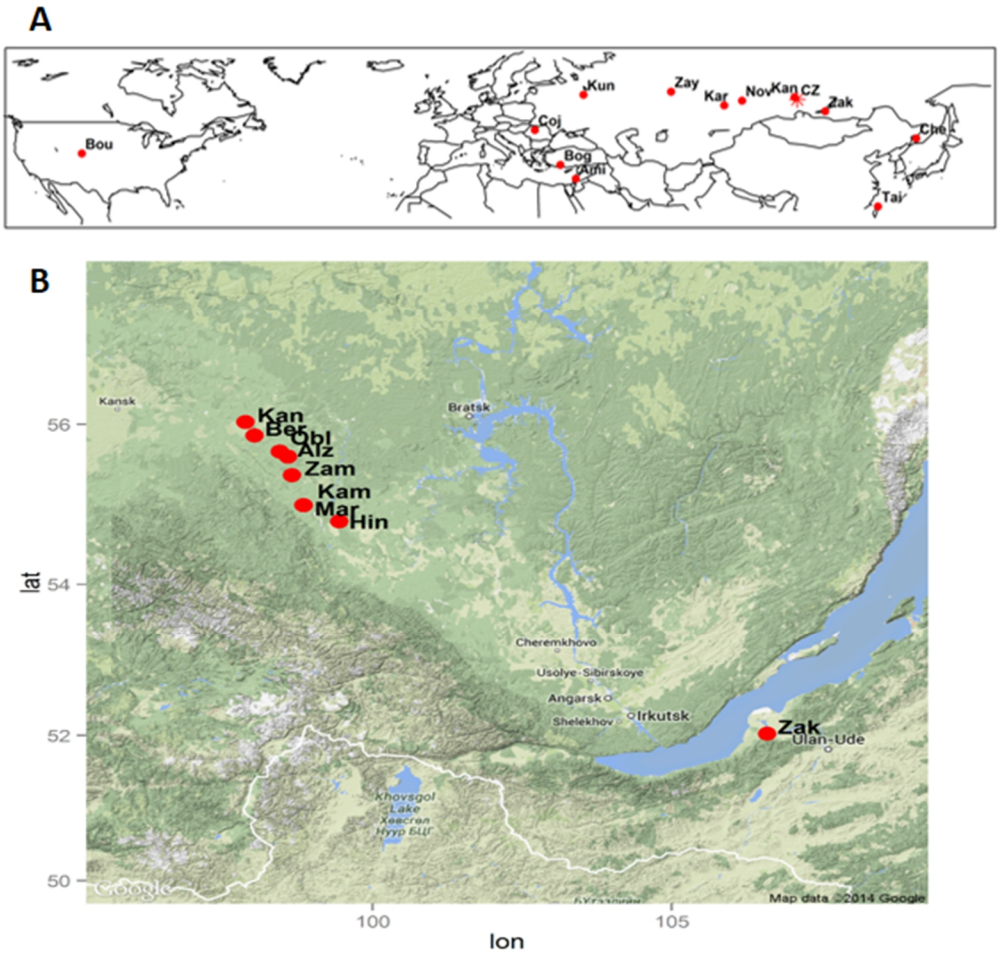

Figure 4.1. Maps of sampling sites; A) shows worldwide sites, denoted by the first three letters of site names (table 1). The asterisk and label 'CZ' refer to the Russian contact zone between *Hirundo rustica rustica* and *H. r. tytleri*. Panel B) shows a terrain map of this contact zone (populations Berezovka-Hingui) and the flanking pure subspecies sites of Kantorksi (*H. r. rustica*) and Zakaltus (*H. r. tytleri*).

Table 4.1. Summary of sampled populations. Means and (standard error) are included for each song parameter

Country	Site	Subspecies	Lat	Lon	N	k	Pulses	WL	RL	R Tmp	PF W	PF R	PF CR	W WE	CR FB
USA	Boulder	erythrogaster	40.10135	-105.2477	66	10.48 (0.62)	10.21 (0.13)	3.08 (0.09)	0.33 (0.01)	31.51 (0.23)	4011.04 (50.54)	4467.14 (52.71)	5468.05 (88.87)	0.63 (0.01)	2650.11 (92.12)
Romania	Cojocna	rustica	46.753192	23.834644	10	12.50 (1.96)	10.60 (0.37)	3.28 (0.13)	0.36 (0.01)	29.65 (0.33)	4154.71 (107.34)	4723.37 (130.59)	5795.84 (127.93)	0.67 (0.01)	2602.70 (289.65)
Turkey	Boğazkent	rustica	36.85771	31.16061	10	9.90 (0.10)	10.53 (0.37)	3.76 (0.20)	0.39 (0.04)	29.37 (0.76)	4378.10 (105.52)	5088.53 (137.44)	5964.06 (106.04)	0.69 (0.00)	3294.36 (238.71)
Israel	Ami'ad	rustica	32.928876	35.540729	11	9.09 (1.33)	11.99 (0.33)	3.24 (0.24)	0.43 (0.01)	27.95 (0.28)	4135.21 (188.04)	5046.06 (269.44)	5980.56 (140.15)	0.66 (0.01)	2011.93 (283.91)
Russia	Kunilovo	rustica	56.731765	37.74458	10	7.00 (1.35)	11.82 (0.41)	2.73 (0.19)	0.41 (0.02)	29.11 (0.53)	4203.44 (125.30)	4461.94 (179.51)	5774.04 (146.72)	0.64 (0.01)	2606.01 (275.76)
Russia	Zaykovo	rustica	57.575132	62.70624	15	7.33 (0.89)	12.42 (0.38)	3.40 (0.19)	0.42 (0.01)	29.59 (0.48)	3881.39 (106.83)	5180.03 (161.33)	5723.90 (113.92)	0.64 (0.01)	2419.30 (241.57)
Russia	Karasuk	rustica	53.730186	77.839711	7	8.43 (1.94)	10.55 (0.37)	3.26 (0.18)	0.37 (0.01)	28.68 (0.44)	4293.09 (174.10)	4554.57 (267.12)	5691.85 (134.97)	0.63 (0.00)	2644.00 (210.97)
Russia	Novosibirsk	rustica	55.031644	82.936521	5	10.00 (1.30)	11.47 (0.63)	3.22 (0.19)	0.39 (0.03)	29.26 (0.55)	4640.83 (56.17)	4483.12 (159.76)	6008.50 (74.51)	0.66 (0.01)	4141.94 (257.34)
Russia	Kantorski	rustica	56.028855	97.876773	3	5.00 (1.00)	12.89 (1.06)	4.05 (0.64)	0.42 (0.03)	30.76 (0.57)	4645.56 (251.18)	5196.11 (485.79)	6005.56 (166.45)	0.64 (0.01)	1981.11 (231.18)
Russia	Berezovka	rustica & tytleri	55.864198	98.026687	2	9.00 (02.00)	10.48 (0.34)	3.19 (0.55)	0.38 (0.00)	27.31 (0.80)	4549.68 (143.96)	5495.39 (171.75)	5920.84 (253.70)	0.64 (0.01)	1857.47 (11.10)
Russia	Oblepiha	rustica & tytleri	55.667468	98.448748	1	8.00 (NA)	11.50 (NA)	2.43 (NA)	0.40 (NA)	29.04 (NA)	4340.00 (NA)	4486.25 (NA)	5820.00 (NA)	0.60 (NA)	2207.50 (NA)
Russia	Alzamay	rustica & tytleri	55.604921	98.58792	2	13.50 (1.50)	09.25 (1.58)	1.80 (0.09)	0.33 (0.02)	27.86 (03.20)	4554.92 (37.58)	410.25 (242.25)	5915.75 (148.25)	0.63 (0.00)	4121.25 (95.42)
Russia	Zamzor	rustica & tytleri	55.37385	98.652222	3	12.33 (04.98)	09.89 (0.79)	2.23 (0.50)	0.39 (0.06)	25.85 (02.52)	4409.02 (138.53)	4746.00 (51.01)	5781.62 (331.81)	0.63 (0.01)	1677.29 (525.36)
Russia	Mara	rustica & tytleri	55.002059	98.845313	5	8.40 (1.63)	12.75 (0.26)	2.72 (0.33)	0.47 (0.02)	27.26 (0.80)	4324.70 (203.74)	5076.14 (236.36)	5904.92 (99.25)	0.64 (0.01)	2525.33 (299.89)
Russia	Kaminka	rustica & tytleri	55.00048	98.848568	1	5.00 (NA)	10.60 (NA)	3.13 (NA)	0.44 (NA)	24.46 (NA)	4416.00 (NA)	4214.00 (NA)	6188.00 (NA)	0.63 (NA)	4870.00 (NA)
Russia	Hingui	rustica & tytleri	54.798431	99.440598	3	5.67 (0.33)	10.99 (1.01)	2.31 (0.09)	0.47 (0.04)	23.39 (0.22)	4456.56 (203.91)	5274.44 (762.72)	6189.11 (112.76)	0.63 (0.01)	2926.44 (870.44)
Russia	Zakaltus	tytleri	52.021259	106.590942	12	8.17 (1.28)	10.46 (0.31)	2.21 (0.14)	0.44 (0.01)	24.01 (0.20)	4614.87 (93.26)	4988.92 (257.31)	5818.12 (95.66)	0.65 (0.01)	2672.99 (205.46)
Taiwan	Taipei	gutturalis	25.041435	121.612744	10	10.50 (1.36)	10.91 (0.37)	2.86 (0.19)	0.36 (0.02)	30.99 (0.47)	3984.66 (60.90)	4987.84 (227.01)	6154.58 (88.53)	0.68 (0.01)	3639.36 (313.18)
Russia	Chernigovka	gutturalis	44.332958	132.517776	4	3.25 (0.25)	11.10 (1.03)	1.86 (0.51)	0.31 (0.03)	35.79 (1.19)	4528.13 (238.04)	5221.88 (399.75)	6246.67 (130.39)	0.66 (0.01)	2429.37 (774.59)

*N= number of males sampled; k= number of songs sampled per male

Table 4.2. Measured song traits and standardized discriminant coefficients

Category	Trait	Description	DF1	DF2	DF3	DF4
Time Domain						
	Pulses	Pulse number—the number of pulses is in the rattle	0.139	-0.620	-0.240	0.056
	WL	Warble length—distance between peak of first syllable and peak of last syllable before P (sec)	-0.243	-0.402	-1.052	0.602
	RL	Rattle length—distance between the first and last pulses in the terminal trill (sec)	-3.426	1.453	3.425	-0.461
	RTmp	Rattle tempo—number of rattle pulses/ rattle length (Hz)	-0.539	-0.019	0.199	0.022
Frequency Domain						
	PF W	Peak frequency of the warble—frequency at the maximum amplitude in the warble (Hz)	0.001	0.001	0.000	0.001
	PF R	Peak frequency of the rattle—frequency at the maximum amplitude in the rattle (Hz)	0.000	0.000	0.000	0.000
	PF CR	Peak frequency of the central rattle—frequency at the maximum amplitude in the rattle, excluding the first and last pulse (Hz)	0.000	-0.001	0.001	0.000
	BW R	Frequency bandwidth of the rattle—song frequency bandwidth above a -10dB threshold, relative to peak frequency, averaged across the entire rattle (Hz)	0.000	0.000	0.001	0.001
	WE W	Wiener entropy of the warble—ratio of the geometric mean to the arithmetic mean of the warble spectrum (0=pure tone; 1=random noise)	-3.285	-14.035	4.483	-13.242

Individual songs were extracted from recording sessions based on recordings spoken into microphone or lapel microphone using Syrinx-PC (J. Burt, Seattle, WA). Barn swallow songs are comprised of a warbling series of syllables, followed by a harsh trill, termed the "rattle" (Galeotti et al. 1997). Although males sometimes utter isolated phrases of syllables, only complete songs, containing a rattle, were considered for this analysis. Song variables (table 4.2) were chosen to measure a large portion of song variation, including frequency and temporal parameters. In addition, Weiner entropy of the warble represents tonality. This measure ranges from 0, representing a pure tone to 1, representing random noise. All song variables were extracted using the Automatic Parameter Measurement function of Avisoft SASLab Pro version 5.2 (www.avisoft.com, R. Specht), except for the number of rattle pulses, which were manually counted from spectrograms. For frequency analysis we measured the rattle, as well as the 'central rattle,' disregarding the first and last pulse. This was necessary because in some barn swallow populations, the frequency of these pulses is much lower than the main pulse train.

We generated spectrograms for automatic parameter measurement in Avisoft (Fast Fourier Transformation = 512, Frame = 100%, Window = Hamming, Overlap = 93.75%), aiming for a balance of frequency and temporal resolution (43 Hz and 1.45 ms, respectively). As in (Wilkins 2014), and unlike previous barn swallow song studies (Galeotti et al. 1997; Garamszegi et al. 2005; Garamszegi et al. 2006a), we did not separate songs and analyses by 'Type A' and 'Type B' songs, as this distinction was not clear across all populations. Because we did not attempt to

classify syllable types or assess repertoire, we set a threshold of 3 complete songs for estimating song parameters, as this was shown to be sufficient in previous rarefaction analysis (Wilkins 2014, figure 8A3.2). Moreover, because structural song traits considered have not been shown to vary predictably over the breeding season (Galeotti et al. 1997, MRW, unpublished data), we did not control for sampling date relative to breeding onset, as this was unlikely to bias estimates of signal divergence across populations.

For this study, subspecies distinctions follow results from Dor et al. (2010), attributing populations sampled in Europe to *Hirundo rustica rustica*, North America to *H. r. erythrogaster*, Israel to *H. r. transitiva*, and eastern Asia to *H. r. gutturalis*. For the Western part of our Russian transect, including the contact zone between *H. r. rustica* and *H. r. tytleri*, subspecies identity of singers was inferred by morphology (*H. r. tytleri* have dark, rusty ventral plumage, in contrast to the pale plumage of nominate *H. r. rustica*) or song (*H. r. tytleri* song is characterized by the lack of a long, rising "p-note" before the terminal trill, which is present in the nominate subspecies, and the presence of a "squawk" following the trill, which is absent in the nominate subspecies). As *H. r. tytleri* are genetically more closely related to North American *H. r. erythrogaster* than sympatric *H. r. rustica* (Dor et al. 2010), and have pronounced morphological divergence, subspecies misidentification is unlikely in this contact zone.

Measuring phenotypic distance:

As a major goal of this study was to determine how song characteristics change with geographic distance in relation to the presence of heterotypic subspecies, we utilized the recently developed 'Δp' nonparametric statistic to derive a measures of phenotypic distance between populations. This statistic has statistical advantages (such as robustness to unequal sample sizes, variances, and scales) over other measures of effect size (such as Hedge's *g*) and was developed explicitly to compare multiple phenotypic traits across multiple populations (Safran et al. 2012). To describe total acoustic difference between each population pair, we calculated Euclidean distance, calculated across Δp values for all nine song measures.

Statistics:

All statistical tests were performed using R v3.0.2 (R Core Team 2014). In order to test the relationships between phenotypic and geographic distance, we used Mantel tests, implemented in the 'ade4' software package (Dray et al. 2007). Further, to identify the song traits which were most important for distinguishing between subspecies, we performed linear discriminant function analysis on centered song variables using the 'MASS' package (Venables and Ripley 2002). The coefficients of each linear discriminant can be interpreted as the magnitude of divergence for traits most important in distinguishing among populations included in the analysis.

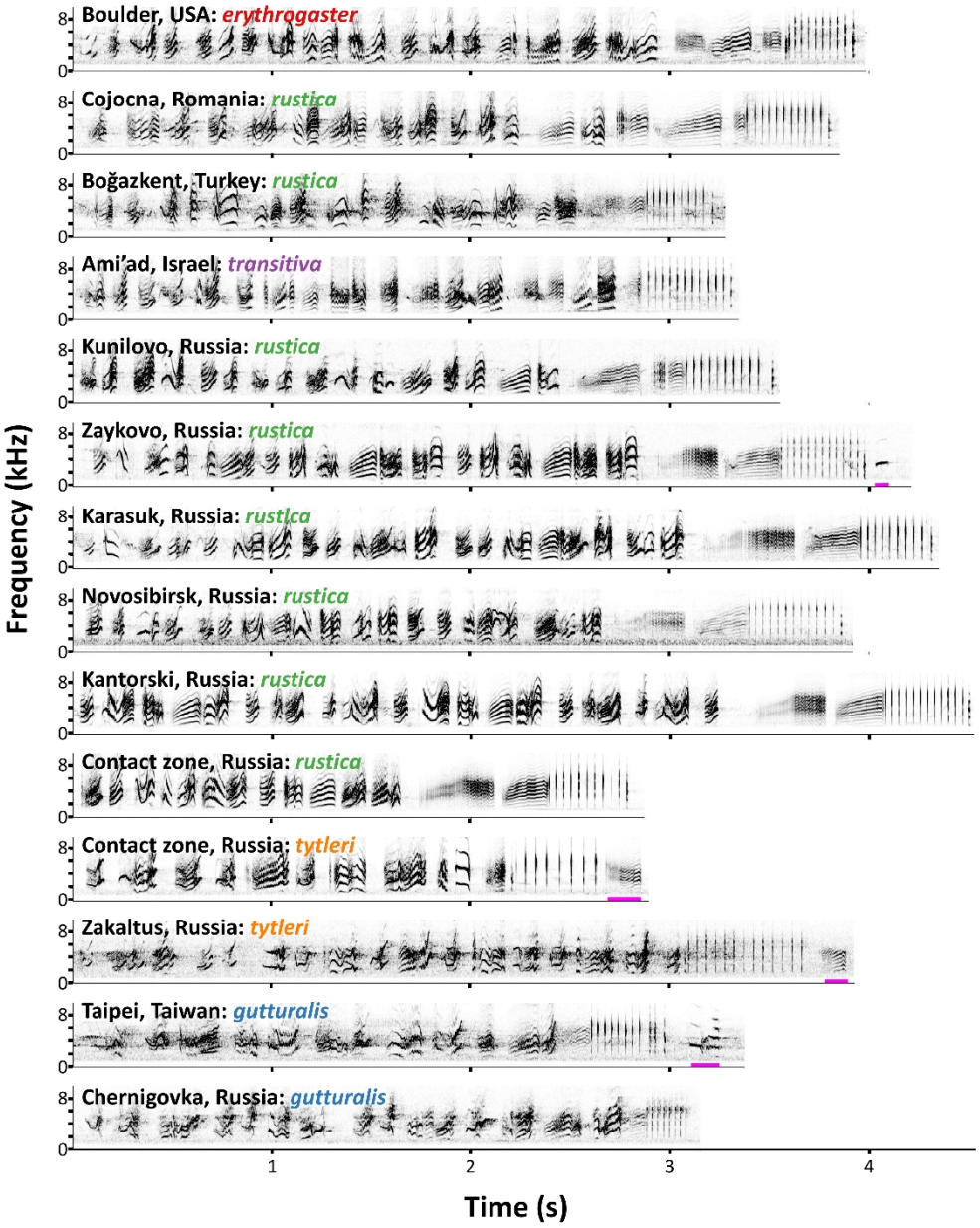

Figure 4.2. Song spectrograms from a single male from each study area. Spectrograms are arranged from West (top) to East (bottom). Songs were chosen based on high signal-to-noise ratios, and the most similar length and tonality across populations. Inferred subspecies is noted after country designations. Because of low sampling, villages within the contact zone were combined for analysis, and only one representative song is included for each subspecies from this region. Pink lines on spectrograms underscore terminal syllables following rattles, which are unique to a particular population (or subspecies, in the case of *H. r. tytleri*).

4.4 Results

Broad patterns

As shown in figure 4.2, the overall structure of song components was remarkably consistent across the 19 sampled populations. Figure 4.3 demonstrates intra-individual song variation for two subspecies, *H. r. tytleri* and *H. r. erythrogaster*. As described previously for the European subspecies (Galeotti et al. 1997), all complete songs consisting of a warbled series of syllables, terminating in a harsh rattle. Some populations produced additional syllables following the rattle, as can be seen for Zaykovo, Russia and Taipei, Taiwan (figure 4.2). These syllables were produced frequently, but not in every bout by a given male, and were sometimes never produced by other males in the same population. In contrast, the terminal 'squawk' (shown in figure 4.3) was a distinguishing feature of *H. r. tytleri* song, and was rarely dropped from songs. In addition, the long, rising 'p-note' (figure 4.2) which was first described in Italy (Galeotti et al. 1997) and is present across the *H. r. rustica* range, is not recognizable in many other populations (figure 4.2; compare Boulder and Ami'ad), and was therefore not measured in this study. In the next sections we discuss which traits are most important for distinguishing subspecies.

Which traits are most divergent?

Our linear discriminant function analysis extracted four functions which accurately classified 75.6% of individuals to subspecies based on song characteristics. Table 4.1 shows coefficients for the four linear discriminants, which explained 66.6%, 19.5%, 12.5%, and 1.4% of the variance, respectively. Standardized coefficients for the first

two most important linear discriminants indicate that higher DF1 scores primarily indicate more tonal songs, with shorter, slower rattles, while higher DF2 scores indicate more tonal songs, with longer, slower rattles (consisting of fewer pulses), and shorter warbles (figure 4.4). From the discriminant coefficients, it is clear that none of the raw frequency measures are important for distinguishing subspecies. Instead, temporal measures, and a measure of tonality are more consistently associated with subspecies distinctions.

Isolation by Geographic Distance?

Pairwise geographic and acoustic differences are reported in Table 4.3. Our results show that Euclidean distances calculated across Δp scores for all song characteristics did not vary consistently over geographic distance, either along our Russian transect or across all populations. Figure 4.5A shows a shallow relationship, with greater acoustic differences accumulating with distance among samples in our Russian transect; however, there was no significant correlation across distance matrices (Mantel test, r= 0.201, p=0.238). There was also no significant relationship in the full dataset (r=0.173, p=0.163), depicted in figure 4.5B. Because we sampled within multiple populations of *H. r. rustica*, *H. r. tytleri*, and *H. r. gutturalis*, these population comparisons are color coded in figure 4.4. This figure highlights a great deal of within-species variation in song traits (e.g. dispersion in green *H. rustica-rustica* comparisons across the graph), with no evidence of isolation by distance.

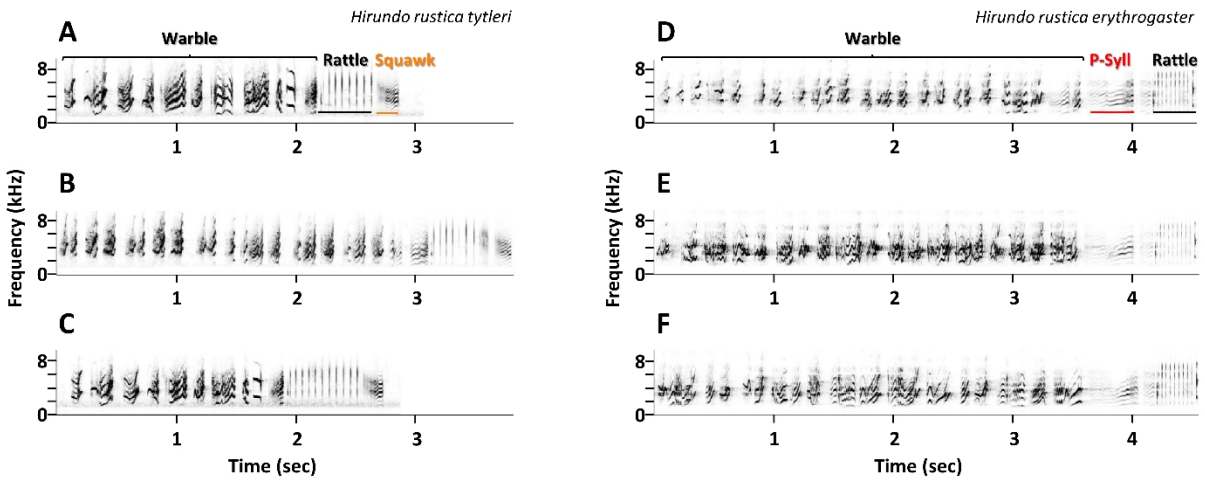

Figure 4.3. Intra-individual song variation. A-C) Spectrograms of three songs from a single male *Hirundo rustica tytleri* from Zamzor, Russia; D-F) spectrograms of three songs from a single male *H. r. erythrogaster* from Boulder, USA. The squawk and p-syllable highlight two song traits which distinguish these two subspecies.

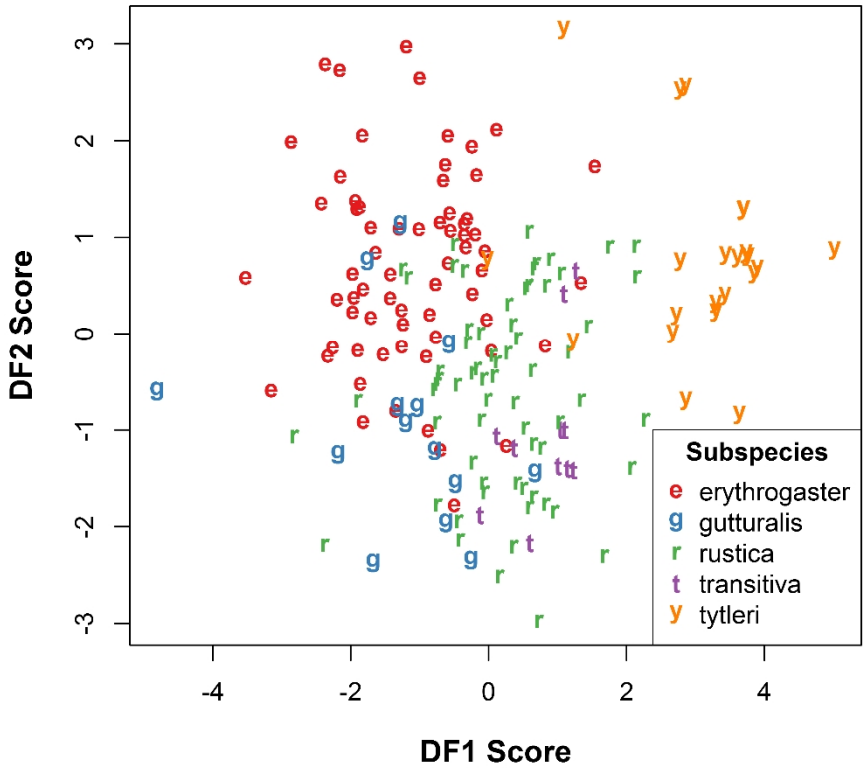

Figure 4.4. Results of linear discriminant function analysis. The x- and y-axes reflect each of 180 individual scores for the first and second linear discriminants, describing differences in the 9 song metrics considered. Different letters reflect different subspecies. Higher DF1 scores primarily indicate more tonal songs, with shorter, slower rattles; higher DF2 scores indicate more tonal songs, with longer, slower rattles (consisting of fewer pulses), and shorter warbles. Thus, *tytleri* songs (orange 'y's) were concentrated on the right half of the graph, and were therefore distinguished by having short, slow rattles; however, they had quite variables.

Table 4.3. Pairwise acoustic and geographic distances between sampling sites

	Bou	Coj	Bog	Ami	Kun	Zay	Kar	Nov	Kan	C_ru	C_ty	Zak	Tai	Che
Boulder_*erythrogaster*		9131	10382	10962	8712	9106	9588	9419	9116	9170	9170	9329	11311	9005
Cojocna_*rustica*	49.83		1257	1831	1462	2864	3825	4120	4989	5061	5061	5694	8576	7724
Boğazkent_*rustica*	93.29	57.84		592	2266	3263	4015	4360	5300	5356	5356	5941	8419	8032
Ami'ad_*transitiva*	112.32	78.32	92.67		2655	3425	4045	4402	5348	5396	5396	5945	8194	8029
Kunilovo_*rustica*	83.99	70.19	96.67	74.68		1502	2530	2777	3586	3663	3663	4304	7345	6292
Zaykovo_*rustica*	82.67	59.56	81.54	54.40	64.09		1039	1276	2126	2197	2197	2830	5849	4871
Karasuk_*rustica*	68.58	54.48	87.33	83.90	46.57	71.10		361	1304	1351	1351	1928	4815	4018
Novosibirsk_*rustica*	94.50	72.39	59.55	109.43	75.69	96.98	70.56		946	997	997	1593	4588	3676
Kantorski_*rustica*	132.14	110.15	101.87	78.37	85.58	91.46	99.05	107.74		88	88	723	3948	2751
Contact_*rustica*	96.57	76.17	90.10	62.22	55.39	63.04	67.79	90.16	92.71		0	642	3861	2687
Contact_*tytleri*	116.35	96.54	113.58	109.42	64.54	107.60	69.76	79.28	128.87	76.48		642	3861	2687
Zakaltus_*tytleri*	116.02	87.86	95.65	99.16	87.00	101.71	86.01	90.63	133.96	60.29	59.27		3261	2092
Taipei_*gutturalis*	85.96	67.33	65.65	104.13	99.01	97.86	99.70	77.13	122.83	100.06	113.50	105.17		2363
Chernigovka_*gutturalis*	104.19	103.78	105.56	126.34	109.63	129.53	120.10	103.06	127.31	106.68	130.20	121.76	72.35	

*Lower triangle values are Euclidean distances between populations across Δp percentile scores for all nine song traits; upper triangle values are geographic distances in km.

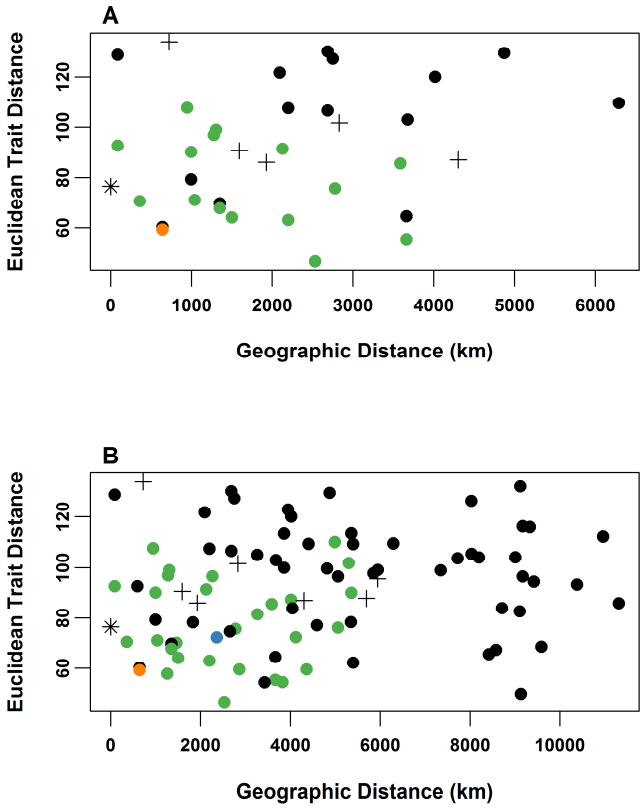

Figure 4.5. Euclidean trait distance versus geographic distance across population pairs, considering all song measures. Panel A) shows only data points collected in Russia, and B) includes all populations. Black points represent inter-subspecies comparisons, with black crosses representing allopatric *rustica*-*tytleri* comparisons; the black asterisk indicates the sympatric *rustica*-*tytleri* comparison. Green, orange, and blue circles represent intra-subspecies comparisons within *rustica*, *tytleri*, and *gutturalis* populations, respectively. Minimum geographic distance is 0 km in the contact zone and maximum distance is 11,300 km, between Boulder, USA and Taipei, Taiwan.

Do songs diverge or converge in sympatry?

With regards to the effect of heterotypic subspecies in sympatry, our results provide evidence for convergence, rather than character displacement. The crosses in figure 4.5B represent *rustica*-*tytleri* population comparisons in allopatry, while the asterisk represents sympatry. From this it is clear that Euclidean phenotypic distance is lower in sympatry than in allopatry. In fact, the nearest allopatric *rustica*-*tytleri* comparison is much more distinct than any of the others, highlighting the convergence in sympatry. This can be explained by the sympatric *rustica* males singing more *tytleri*-like songs. As shown in figure 4.5, *rustica* songs had shorter warbles and slower rattles, which converge on *tytleri* means for these traits. The decrease in trait values between Kantorski *rustica* and contact zone *rustica* was significant for rattle tempo (Welch's t-test, assuming unequal variances:

t=3.94, df=4.32, p=0.015), but not for rattle length (t=-0.078, df=3.90, p=0.942), warble length (t=1.65, df=2.68, p=0.208), or warble Wiener entropy (t=0.867, df=2.74, p=0.455).

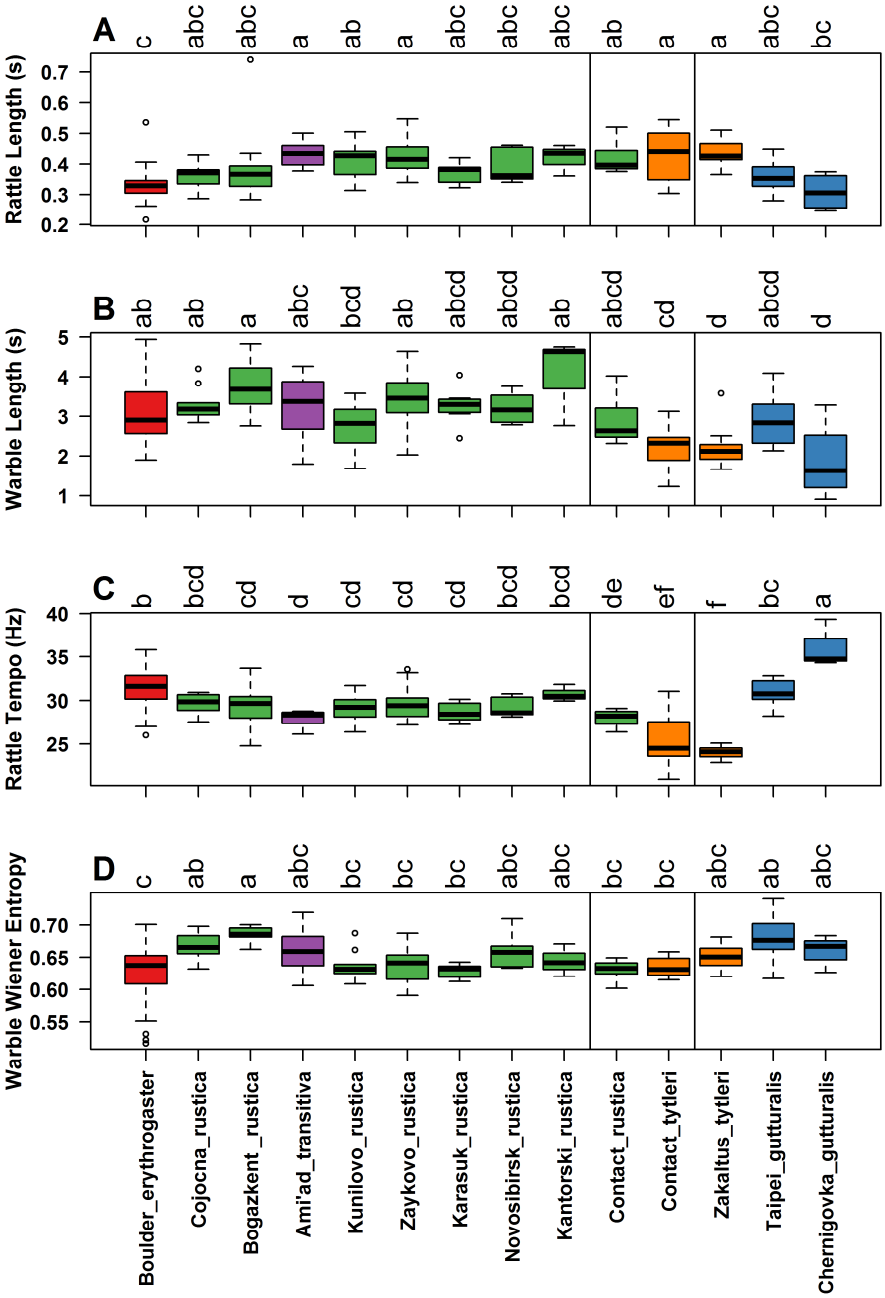

Figure 4.6 Population differences for four traits important for distinguishing subspecies songs. Panels show differences in A) rattle length, B) warble length, C) rattle tempo, and D) Wiener entropy of the warble. Red= H. r. *erythrogaster*, green= H. r. *rustica*, purple= H. r. *transitiva*, orange= H. r. *tytleri*, and blue= H. r. *gutturalis*. The vertical lines indicate populations sampled in the *rustica*-*tytleri* contact zone, and different letters above boxes denote significant differences at the 0.05 level for p-adjusted, accounting for multiple comparisons.

4.5 Discussion

This study represents the first description of song divergence within the barn swallow species complex. We demonstrate broad conservation of song syntax across 19 sampled populations spanning the Holarctic distribution of this species. There was substantial divergence in the nine measured song traits within and across subspecies populations. Temporal traits (the length of warbles and the speed and length of rattles), and a measure of tonality (Wiener entropy of the warble) were the four traits which were most important for distinguishing subspecies song. None of the direct frequency measures included in our study loaded strongly in discriminant function analysis, as these traits vary widely within and across populations, but fall within an overlapping range which may represent species physiological limits. This result is consistent with other recently diverged taxa, such as *Laupala* crickets (Mendelson and Shaw 2005), antbirds (Seddon and Tobias 2007), wrens (Toews and Irwin 2008), and cichlids (Amorim et al. 2008), which are most easily distinguished by temporal traits. Because frequency characteristics are often tied to body mass and size of acoustic signaling structures (Gillooly and Ophir 2010), while the rate or length of acoustic signal production is likely subject to fewer constraints, variation in temporal traits may be a common distinguishing feature between recently diverged populations.

Unlike some previous studies of birdsong (e.g. Irwin et al. 2008), we did not find evidence for isolation by distance in song divergence. There was no significant correlation between geographic and Euclidean acoustic distance for our global

sample or our Russian subsample. This result implies that deterministic forces, such as sexual or ecological selection outweighed gradual accumulation of song differences over space. As an example of how context-specific selective pressures affect signal divergence, we found that *H. r. rustica* songs became more similar to *H. r. tytleri* in sympatry. Specifically, males produced significantly slower rattles, which contrasted strongly with the nearest allopatric *H. r. rustica* population. These findings are similar to those for South American antbirds, suggesting that signal convergence may be favored under certain conditions (Tobias and Seddon 2009). As *H. r. rustica* and *H. r. tytleri* are among the most genetically distinct subspecies in the barn swallow complex, it is possible that selection favors increased similarity, without deleterious effects on increased hybridization. However, the degree to which the observed pattern is explained by phenotypic plasticity is unknown, and whether signal convergence results from selection to improve performance in heterotypic agonistic interactions or increase the probability of attracting heterotypic mates is an outstanding question for future work. We hope this study will stimulate increased interest in barn swallow song divergence, and provide the groundwork for future research on the local selection processes driving broad patterns of geographic variation in acoustic signals.

CHAPTER 5

THE ROLE OF TRILL RATE IN BARN SWALLOW COMMUNICATION: MALE-MALE COMPETITION, REPRODUCTIVE SUCCESS, AND SIGNAL INTERACTIONS [4]

5.1 Abstract

A common class of acoustic signals, trills, are composed of simple, rapidly repeated elements, and utilized by orthopterans, anurans, and oscine passerines for mate choice and competition. Due to the difficulty of their production, trills may have evolved as "index signals" which honestly indicate an individual's quality or fighting ability. Trill rate is highly variable across barn swallow subspecies, potentially due to differential selection on trill rate among populations. We used a North American population of barn swallows *H. r. erythrogaster* to perform simulated territorial intrusions using taxidermic mounts paired with song stimuli to test the role of trill rate in barn swallow communication. Previous work on swamp sparrows suggests that males respond to competitor's trill rates based on their own trill rate. In contrast, we found that resident male trill rates did not predict response level to normal or artificially enhanced trill rate treatments. Instead, resident males responded more strongly to enhanced trill rates only when the intruder was a darker color. Furthermore, males which took longer to respond to the enhanced stimulus compared to the normal stimulus fledged more offspring, though this did not translate to increased genetic paternity. Collectively, these results highlight the importance of considering intra- and intersexual selective perspectives, as well as interactions across signaling modalities (here, visual and acoustic) in studies of the evolution of animal communication systems.

---

[4] This work was undertaken in collaboration with M. D. Merz and R. J. Safran.

5.2 Introduction:

Acoustic signals are important for mediating social interactions in a broad range of taxa. One common class of signals, trills, are composed of simple, rapidly repeated elements, and utilized by orthopterans, anurans, and oscine passerines for mate choice and competition (Naguib 2003). In birds and frogs, the rate of element repetition is limited by mechanical movement of the vocal tract (Schmidt 1965; Westneat et al. 1993). Therefore, due to the difficulty of their production, trills may have evolved as "index signals" which honestly indicate an individual's quality or fighting ability (Searcy and Beecher 2009). In support of this, trill characteristics have been linked to sexual selection via female choice (Vallet et al. 1998; Ballentine 2004), intrasexual competition (de Kort et al. 2009), or both (Sprau et al. 2010). Further, a recent experimental study in swamp sparrows demonstrated that receiver attributes predicted responses to playback stimuli which varied in trill rate. That is, naturally fast-trilling males responded more aggressively than slow-trilling males to simulated territory intrusions (Moseley et al. 2013). This finding suggests that competing males assess rivals based on trill rate and modulate responses based on their own relative quality. However, birds commonly use both visual and acoustic cues in competition, and it is currently unclear whether trill characteristics interact with other aspects of morphology to mediate agonistic interactions between competing males.

Trill rate represents one of the most salient features of barn swallow song distinguishing among subspecies (Chapter 4, this dissertation). To assess the social function of this trait in communication, we experimentally investigated the role of

trill rate in mediating intrasexual competition and reproductive outcomes within a population of *H. r. erythrogaster*. We performed a two-year song playback experiment at various breeding sites in a Colorado, USA study population. We simulated territory intrusions on resident male barn swallows using taxidermic mounts, paired with two song stimuli: a "normal" song bout and a "fast" song bout, with artificially sped-up trills. By utilizing taxidermic mounts, we were further able to assess how intruder morphology, relative to that of residents, affected response behavior. In addition, we monitored reproductive success throughout the breeding season, collecting both fledging success and genetic paternity for focal males. This study design allowed us to 1) assess how males responded to the two experimental treatments focused on the trill rate, accounting for individual behavioral variation, 2) determine which phenotypic factors of resident and intruder explain variation in behavioral responses, and 3) quantify how resident-intruder dynamics relate to social and genetic measures of fitness. The overall goal of this study is to assess the role of trill rate in both intrasexual- and intersexual selective contexts in order to improve our understanding of how this broad class of acoustic signals functions in communication.

Barn swallows (*Hirundo rustica*) are semicolonial, migratory oscine songbirds, comprising six described subspecies distributed across the Holarctic. Recent and classic work among various worldwide populations demonstrates that different targets of sexual selection have led to rapid phenotypic and genetic divergence across populations (Møller 1994; Safran et al. 2005; Neuman et al. 2007; Hasegawa et al. 2010; Vortman et al. 2011; Vortman et al. 2013; Safran et al.,

unpublished manuscript) within the last 100,000 years (Zink et al. 2006; Dor et al. 2010). Moreover, a recent study of song divergence demonstrated broad conservation of syntax (the overall structure of songs) throughout the barn swallow species range (Chapter 4, this dissertation). Songs are typically composed of a warbling series of syllables, leading to a terminal trill, termed the "rattle" (Galeotti et al. 1997) (shown in figure 5.1B). However, the identity and arrangement of syllables in the warble is highly variable within and among populations, and the temporal patterning of song components was shown to differ to a greater degree than frequency traits across subspecies (Chapter 4, this dissertation). In particular, the rate of pulse production in the rattle ("rattle tempo") showed pronounced differentiation, with the Colorado study population exhibiting among the fastest rattle tempos sampled (Figure 4.5). Previous work in an Italian population demonstrated that rattle length increased with the number of competing males, corresponded with testosterone levels (Galeotti et al. 1997), and increased in response to artificial elongation of tail streamers (Saino et al. 2003), which are a sexually selected signal within the European subspecies *H. r. rustica* (Møller 1994). Moreover, rattle tempo strongly correlated with pairing success in a Spanish population, indicating the rattle may relate to female choice, in addition to intrasexual competition (Garamszegi et al. 2006a). Because rattle length and tempo are negatively correlated within populations (Chapter 3, this dissertation), they may represent a physiological tradeoff akin to that between frequency bandwidth and trill rate, demonstrated for numerous bird species (Podos 1997; Podos 2001; Ballentine 2004; Wilson et al. 2014). Thus, selection for elaboration in one of these

dimensions at the cost of the other may allow for rapid signal divergence across populations in sexually selected song components.

Within North American *H. r. erythrogaster*, previous work demonstrates that males with shorter distances to the nearest competing male had shorter, faster rattles, indicating the importance of rattle tempo in mediating male-male competition (Chapter 3, this dissertation). Further, rattle tempo (along with tightly correlated rattle length and syllable tempo) was also an important factor in determining genetic paternity, a surrogate for female choice in barn swallows, in which forced copulation is extremely uncommon (Møller 1985). Together with the fact that rattle tempo within this population is among the highest reported (Chapter 4, this dissertation), these factors make the North American barn swallow an ideal study system to investigate the role of trill rate in sexual communication.

5.3 Methods:

Field Methods:

This study was conducted in 2011 and 2012 in Boulder County, Colorado, USA (Latitude 40° 29' 360" N, Longitude 105° 169' 390" W). Eight study sites were used, and each had between 3 and 43 nesting pairs. Each year, we began running trials as soon as males arrived and began actively defending territories. Experimental trials were conducted between May 6-31, 2011 and May 10-29, 2012, from 5:30 to 11am. Because trials sometimes preceded banding efforts, males were identified by distinguishing morphological features, the presence of a previous year's color band,

or by territory. As soon as possible, barn swallows were captured using mist nets, banded with USGS metal bands, given a unique combination of a color band and nontoxic permanent ink applied to white spots on rectrices; thus, individual identity could be confirmed and tracked in association with a particular social mate and nest site. The length of streamers (the outermost tail feathers) was measured upon capture. In addition, contour feather samples were taken for color analysis. Blood samples were taken from adults upon capture and from nestlings on day 12 post-hatching for paternity analyses. During the breeding season, we matched banded individuals to nests and monitored reproductive success for all active nests at study sites. All methods described herein were approved by the University of Colorado Institutional Animal Care and Use Committee (Protocols 07-07-SAF-01 and 1004.01).

Stimulus Preparation:

Twenty-seven trial stimuli were created from separate individuals recorded from the Colorado population in 2009. Of 53 trial attempts, 31 were successful for both treatments. Twenty-two of twenty-seven stimuli were used in successful trials, and

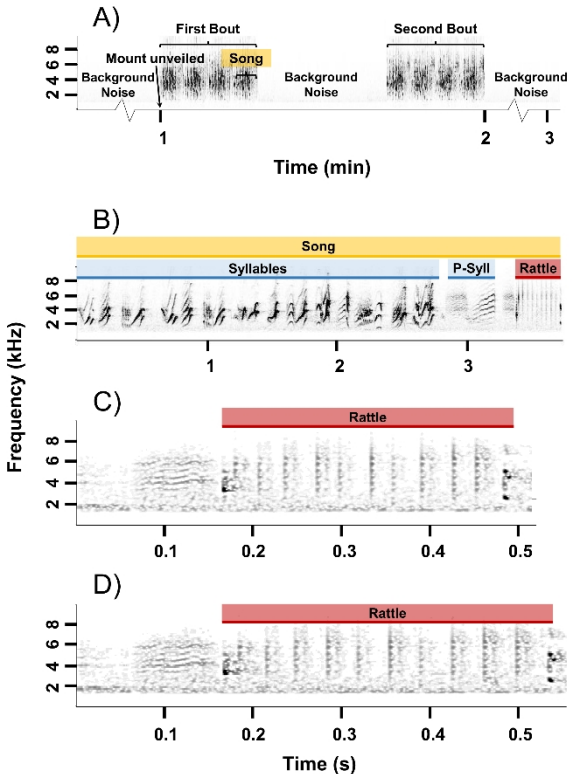

Figure 5.1. Elements of experimental design. Panel A) shows a schematic stimulus setup, artificially created from one "normal" song or a "fast" song, with the rattle sped up 15%; B) depicts a sonogram, with the primary subunits of male H. r. erythrogaster song; C) shows a "fast" rattle, which has been artificially sped up; and D) shows a "normal" rattle.

nine of these were reused across years. However, no stimulus was reused in the same year, and the minimal potential for pseudoreplication (Hurlbert 1984; Kroodsma et al. 2001) across years was controlled for within our random effect 'Year' in our mixed models. Paired "fast" and "normal" stimuli were created from each song using Adobe Audition 3. Each stimulus was three minutes long. The first minute was brown noise (level 16 was typically used, but was visually adjusted to match the background noise of recordings used for stimuli, to control for the effects of noise alone on behavior). The second minute was a playback, created by copying the same song 8 times, to form a bout of four adjacent songs at the beginning of the period, and a second bout of four at the end, with background noise between bouts. This song bout structure is typical of males in a high state of excitement. Although we constructed bouts from a single song recording, while males almost never repeat the same sequence of syllables in a warble (Galeotti et al. 1997; MRW, personal observation), this design was necessary to control for the high variability of song composition within song bouts and isolate the effects of rattle tempo on behavior. The third

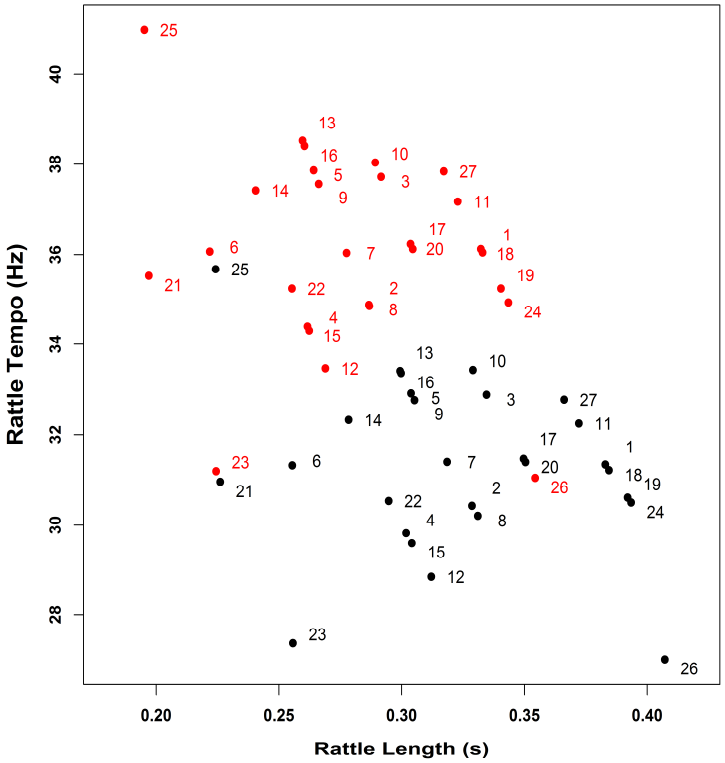

Figure 5.2. Relationship between rattle length and tempo for the 27 song stimuli. Numbers refer to individual stimuli, with black and red colors representing "normal" and "fast" stimuli, respectively.

minute of each playback was the same background noise as the first. “Fast” stimuli were exactly the same as “normal” stimuli, except that the rattles were sped up by 15% using the Stretch function of Audition. Figures 5.1C and 5.1D illustrate the difference between treatments. Figure 5.2 further shows the changes in rattle length and speed for each stimulus pair, demonstrating that the 15% increase in rattle tempo (red dots), just overlaps with the natural distribution of phenotypes (black dots). Thus, this seemingly subtle change in the overall signal represents the boundary of natural possibility for this population’s song, and should represent a very high quality and/or motivated competitor.

Experimental Design:

We built an apparatus for simulating territory intrusions for resident male barn swallows inside barns (figure 5.3). A Rubbermaid box was attached via hinges to a wooden platform, which was secured to a camera tripod via a 1/4” insert nut installed on the underside of the lumber. The box could be opened from a distance by a string attached to the side opposite the hinges. In the center of the platform, a 3/8” bore allowed for the placement of one of three barn swallow taxidermic mounts, positioned on dowel perches. Taxidermic mounts were prepared by MRW and MM. A hole cut in front of the bore allowed an Altec Lansing Orbit Speaker (frequency response: 100Hz~20kHz) to sit flush with the platform, projecting playback stimuli upward from beneath the bird mount. For trials, we placed the apparatus 1m in front of and approximately 2m below a resident’s primary singing perch which we had previously identified. We then observed a resident male from a makeshift hide to ensure normal behavior and attempted to record spontaneous song prior to

beginning a trial. As shown in figure 5.1, each trial consisted of three periods: a one minute pre-trial observation period, a one minute stimulus period, and a one minute post-trial observation period. The stimulus period began when we pulled the string, opening the box, and simultaneously initiated a playback stimulus using a Sansa 8Gb Fuze portable music player, connected to the speaker via a 20ft 1/8" audio extension cable. Stimulus volume was standardized to approximately 69dB average and 70dB maximum (A-weighted), integrated over a 3s period using a Radioshack digital sound level meter, measured at one meter distance. Each male received two treatments of a randomly selected stimulus pair, presented in randomized order. Trials consisted of a "normal" stimulus, created from a single song recording of a spontaneous song or a "fast" stimulus, using the same song, but with rattles artificially sped-up. Song stimuli were prepared from different males, recorded in 2009, and it is unlikely that individual identity of stimulus singers played a role in responses. After a successful trial, we reset the playback apparatus, and waited at least twenty minutes for a male to resume normal behavior. All trials were videotaped using HD camcorders, and male vocalizations were also recorded, using a parabolic microphone. MRW verbally annotated trial response behaviors for later data extraction by speaking into a lapel microphone. Synchronization across videos and audio recordings for precise

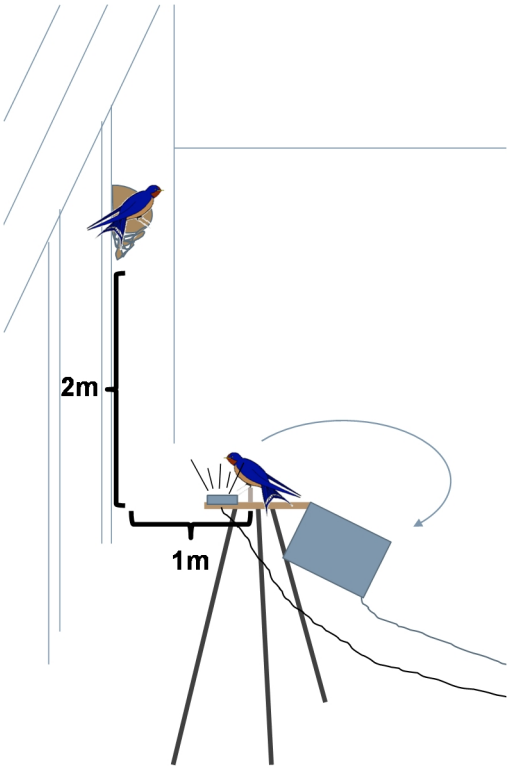

Figure 5.3. Schematic of playback apparatus and simulated territorial intrusions.

extraction of data was accomplished by the presence of a low-frequency audible tone played at the beginning of the pre-trial observation period.

Phenotypic Measurements:

We measured four key traits of resident males and their simulated intruders (shown in table 5.1). These traits were selected due to previous work indicating the importance of these signals in sexual communication within this system. Similar to Safran et al. (2010), we calculated average brightness, hue, and red chroma from sampled ventral feathers. In that study, feathers were sampled from four patches (throat, breast, belly, and vent). However, because previous work indicated strong correlations between all measures of color, across all feather patches implicated in female choice (Chapter 3, this dissertation), we chose to use breast average brightness as a reliable measure of ventral color. In addition, because breast and belly hue were implicated in male-male competition, rather than female choice (Chapter 3, this dissertation), and these chromatic color metrics are not strongly correlated with other color metrics, we included breast hue as a second measure of color.

In addition, between three and 19 spontaneous songs (8.44 (mean) ± 0.897 (SE)) were recorded for 18 males. Recordings were captured in 16-bit WAV format, with 48kHz sampling rate using a Marantz PMD 660 digital recorder, paired with an Audiotechnica AT815B shotgun microphone (2009), a Marantz PMD 660 paired with a Sennheiser MKH 20 and Telinga parabola (2011), or a Marantz PMD 661 paired with a Sennheiser ME62/k6 microphone and Telinga parabola (2012). Songs were recorded between fifteen days and a few minutes prior to trial onset, except for

two males, who were recorded 24 and 97 days after the trial, respectively. We have previously found no relationship between rattle tempo and recording date or breeding status (Wilkins, unpublished data), and trials were unlikely to affect behavior at this timescale. Thus, we did not correct for differences in sampling date for this song trait. Rattle tempo measurements of individual males and stimuli were made using Avisoft SASLab Pro version 5.2 (www.avisoft.com, R. Specht). Spectrogram parameters were as follows: Fast Fourier Transformation= 512, Frame= 100%, Window= Hamming, Overlap= 93.75%, frequency resolution= 43Hz, temporal resolution 1.45 ms.

Analysis of playback response

As measures of response to our song stimulus treatments, we calculated the latency (time delay) to move, vocalize, and/or sing in response to the onset of the stimulus portion of a trial. All but one male demonstrated one or all of these behaviors, and this male was maintained in the dataset, as visual observations indicated the male attended to the stimulus, and his silence and lack of movement accurately reflected his response (or nonresponse, in this case). Although many bird species will attack a speaker or peck at coarse approximations of rival males, barn swallows perform only comparatively subtle responses to simulated intruders. Thus, we feel these approximations capture enough behavior to inform us on the perceived threat of an intruder. Specifically, we expected that males would have lower latencies (would move, vocalize, or sing sooner) when faced with a high quality "fast" intruder. To facilitate interpretation, we performed principal components analysis to reduce our three behavioral response variables to a single principle component, which we

hereafter denote as [Response]. Following varimax rotation, [Response] had an eigenvalue of 1.28, and explained 43% of the variance. Higher [Response] values indicate longer latencies to vocalize, sing, and to a lesser degree, a longer latency to move following the onset of the stimulus portion of trials. Thus, a higher response score indicates that a male was slower to behaviorally respond to the stimulus. Response variable descriptions and loadings on [Response] are shown in table 5.1.

Paternity Analyses:

As extra-pair copulation is relatively common in barn swallows (Saino et al. 1997c), and in order to inform our understanding of how our experimental results relate to apparent versus genetic reproductive success, we assigned paternity to offspring in each focal male's nest using six polymorphic microsatellite markers. We analyzed allele frequencies and performed paternity exclusions using CERVUS 3.0 (Kalinowski et al. 2007). None of the six loci deviated from Hardy-Weinberg equilibrium. The probability of correctly excluding a focal male as the genetic father was 0.9891, and given a known mother was 0.9991. Overall rates of extra-pair paternity in nests were 27.8%, comparable to rates found in other *H. r. erythrogaster* populations (range: 23-34%) (Kleven et al. 2006; Neuman et al. 2007; Eikenaar et al. 2011). For further details on our methods for paternity analysis, see Chapter 3.

Statistical Analyses:

All statistical analyses were conducted using R v3.0.2 (R Core Team 2014). To control for considerable variation in nest density, habitat quality, and other factors across sites and years, we utilized general and generalized linear mixed models (LMM and GLMM, respectively). These were specified using the 'nlme' (Pinheiro et al. 2013) and 'lme4' packages (Bates et al. 2014).

5.4 Results:

Male Responses to Treatments:

We predicted that males would have a lower [Response] (shorter latency to act) when confronted with a "fast" stimulus, compared to a "normal" stimulus. However, as shown in figures 5.4A and 5.4B, there is no relationship between the rattle tempo of a focal male and his [Response] for the normal (Linear Mixed Model (LMM),

Table 5.1. Behavioral response variables extracted from trial annotations, and loadings of these variables on the composite [Response] variable. A lower [response] indicates a shorter time to vocalize, sing or move in response to the stimulus presentation.

Variable	Description	Loading on [Response]
Latency to Vocalize	Time from the opening of box and onset of playback to the first vocalization from resident male	0.70
Latency to Sing	Time from the opening of box and onset of playback to the first song from resident male	0.75
Latency to Move	Time from the opening of box and onset of playback to the first flight from resident male	0.47

random effects=site in year: n=31, t=-0.697, p=0.4941) or the fast treatment (LMM: n=31, t=-0.449, p=0.659). This can be understood by examining figure 5.5, in which there is no consistency in the reaction norms of male responses across treatments.

This result could be due to individual males reacting differently to each treatment, depending on their perceived quality, relative to the intruder. However, figures 5.4C and 5.4D show male [Response] values as a function of each stimulus trill rate, relative to their own intrinsic trill rate. Yet, again, there was no significant relationship for the normal (LMM: n=18, t=1.288, p=0.217) or the fast treatment (LMM: n=18, t=0.354, p=0.731).

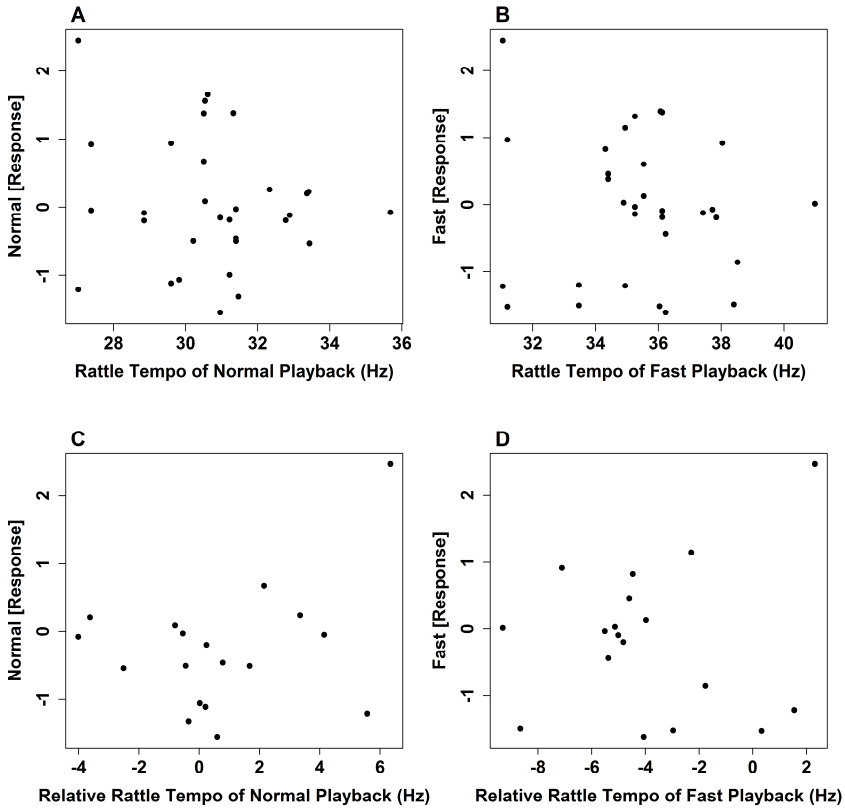

Figure 5.4. Male [Response] values as a function of A) the trill rate of the "normal" playback; B) the trill rate of the "fast" playback; C) the trill rate of the "normal" playback, relative to the resident's trill rate; and D) the trill rate of the "fast" playback, relative to the resident's trill rate. No relationships are significant from linear mixed models, including site and year as random variables.

Predictors of Responses Across Treatments:

It is at first counterintuitive that males would not react more quickly and engage with a more threatening competitor, even when accounting for a male's own rattle tempo, relative to the intruder. However, further insight may come from examining how a male's phenotype relative to the intruder predicts the difference in his behavior across treatments. That is, how each male's behavior changes across

treatments could indicate his strategy in dealing with a normal versus a strong (fast-trilling) competitor. Furthermore, this difference in responses may be a function of other aspects of a resident's phenotype, relative to the intruder. Thus, consideration of visual signals may highlight important interactions across modalities. Figure 5.6 shows differences in response to the fast minus the response to the normal stimulus as a function of rattle tempo, tail streamer length, breast hue, and breast brightness, each measured relative to the intruder. Surprisingly, once again difference in rattle tempo does not predict change in behavior across treatments (LMM: n=18, t=-0.724,p=0.487), nor do differences in tail streamer length (LMM: n=30, t=-0.510, p=0.616), or breast hue (LMM: n=29, t=-0.665,

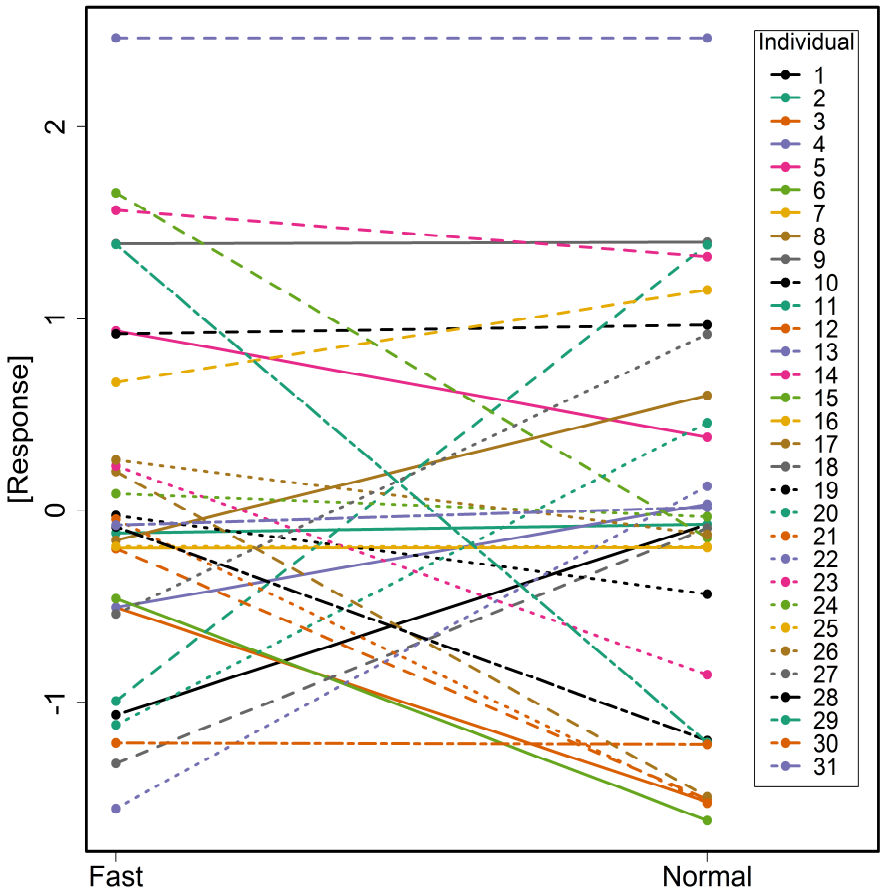

Figure 5.5. Responses of individual males across treatments. Different color/line type combinations reflect different males. Higher response values indicate a longer latency to sing, vocalize or move. The purple dashed line at top indicates a male that did not perform any of these actions across treatments.

p=0.514). Instead, males who were intruded upon by a darker male than themselves (lower breast brightness) engaged more quickly with the fast than the normal stimulus (LMM: n=29, t=-2.96, p=0.008). That is, there was a significant interaction between the darkness and rattle tempo of the intruder on a resident's latency to react.

Intruder Assessment Dynamics and Fitness

Given that we know males utilize trill rate in agonistic interactions (Chapter 3 and this study), and females may also utilize this signal in mate choice (Chapter 3), the ability to dynamically assess and respond to threats signaled by competitors with different trill rates may itself carry evolutionary benefits. Figure 5.7A shows a linear relationship between difference in [Response] across treatments and the number of offspring fledged over the breeding season (Generalized Linear Mixed Model (GLMM), family=Poisson, random effects= site in year: n=29, z=2.15, p=0.032). In contrast, as is clear from figure 5.7B, there is no relationship between difference in [Response] across treatments and the proportion of within-pair young (GLMM, family=Binomial, random effects= site in year: n=24, z=0.267, p=0.790).

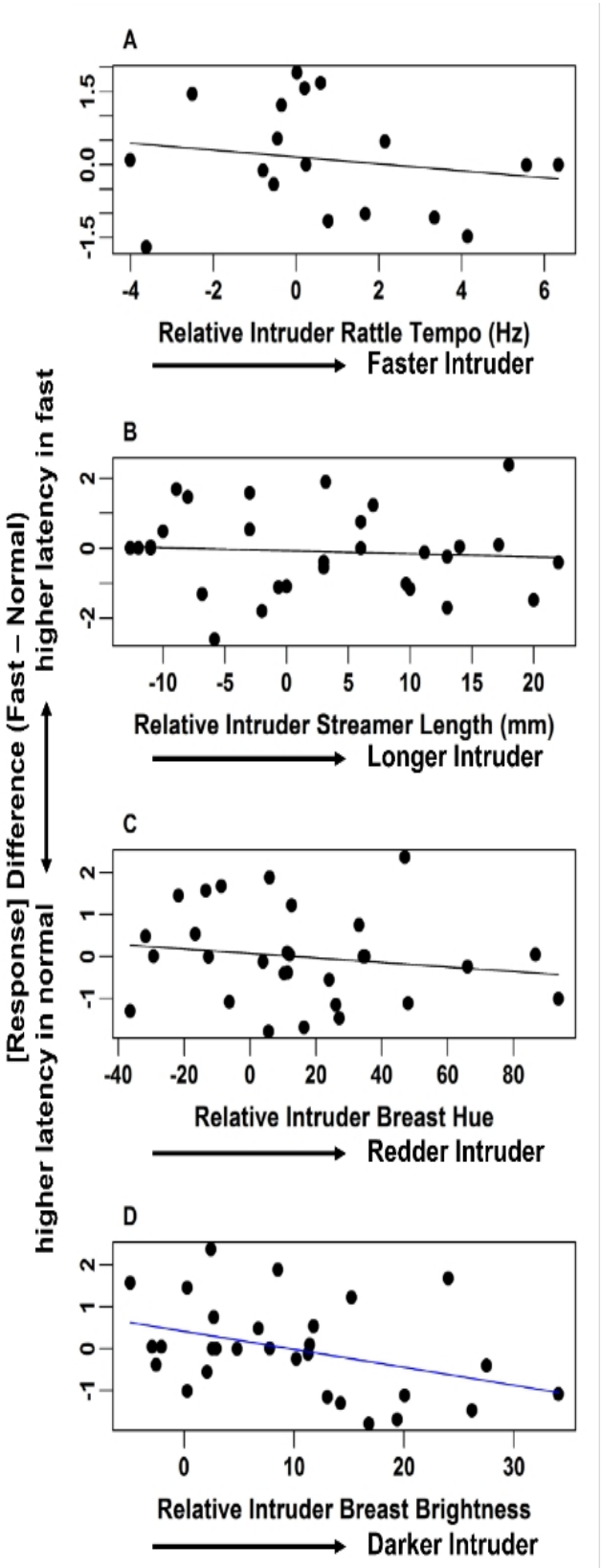

Figure 5.6. Relationships between relative intruder phenotype (resident minus intruder value) and difference in response across treatments ("fast" minus "normal" value). Panels show difference in response across treatments as a function of A) relative intruder rattle tempo (comparing resident unsolicited song rattle tempo to "normal" stimulus rattle tempo), B) relative intruder streamer length, C) relative intruder breast hue, and D) relative intruder breast brightness. Linear regressions are shown, with blue indicating a significant relationship in a linear mixed model.

5.5 Discussion:

Male responses to trill rate enhancement

Males only showed a consistent tendency to respond sooner to a faster trilling intruder when that intruder had darker breast color than the resident. As experimental and correlative studies have shown that dark ventral plumage is a sexually selected trait within *H. r. rustica* (Safran et al. 2005; Neuman et al. 2007; Eikenaar et al. 2011), this result suggests that rattle tempo and color are reinforcing signals of quality. That is, this high quality song trait is only attended when signaled in combination with a high-quality visual trait (here, darker ventral plumage). A similar result within the European subspecies *H. r. rustica*, where males with the longest tail streamers have greater reproductive performance, indicates that only males with long streamers derive increased genetic paternity

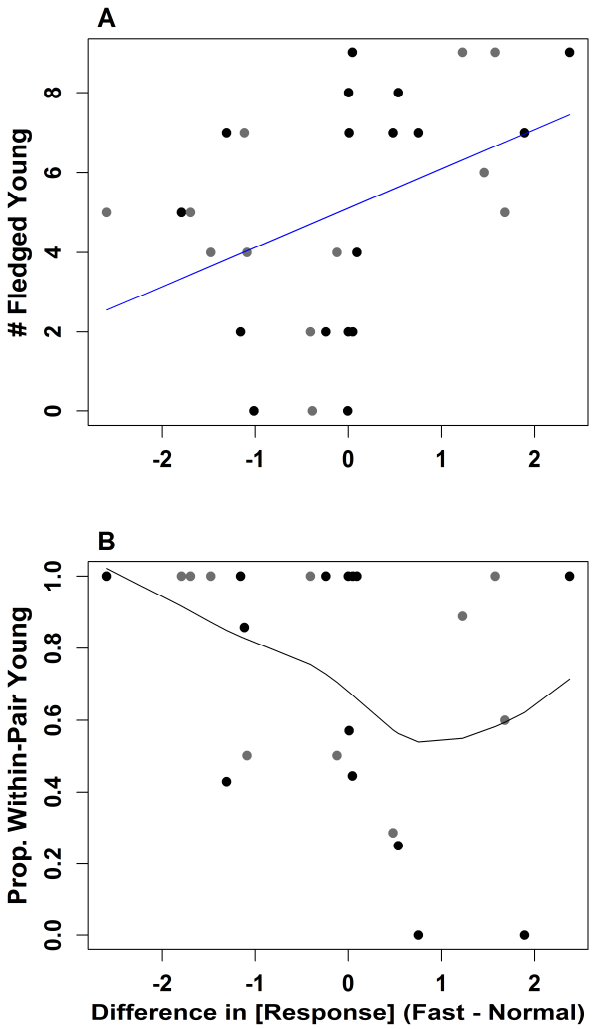

Figure 5.7. Relationships between [Response] differences across treatments and A) the number of young fledged from a resident male's nest over the breeding season, and B) the proportion of genetic offspring sampled on day 12 post-hatching in a focal male's nest. Black dots were sampled in 2011 and gray dots were sampled in 2012. The blue line indicates a significant relationship in a general linear mixed model. A cubic spline is shown for Panel B, and the binomial generalized linear mixed model was not significant for proportion of within-pair offspring.

from increased song rate (Møller et al. 1998). In this case, females prefer males singing at high rates only when these males also had long streamers. In both cases, a visual and an uncorrelated song trait interact to affect receiver behavior. It is possible that song acts as a long distance signal of quality, while color or streamer length provide further information in close proximity (Uy and Safran 2013). This scenario would provide both competing males and choosy females "multiple messages" (Møller and Pomiankowski 1993; Johnstone 1996) for discriminating among competitors and potential mates. However, divergence in the targets of sexual selection across subspecies has led to the importance of tail streamer length in *H. r. rustica* and breast color in *H. r. erythrogaster*. Song rate was not considered in our study, and we cannot assess relationships between this song trait and color for our population. However, experimental elongation of *H. r. rustica* males was shown to increase rattle length, with no effect on song rate (Saino et al. 2003). Thus, rattle length may reinforce sexually selected streamer length in agonistic interactions within European *H. r. rustica*, while rattle tempo reinforces sexually selected ventral brightness within North American *H. r. erythrogaster*. Future comparative experiments are necessary to test this possibility.

Territory defense and fitness

Our findings on reproductive benefits associated with behavioral responses across trials highlight an interesting distinction between apparent and genetic reproductive success. The fact that males with a longer latency to respond to a "fast" versus a "normal" intruder fledged more offspring may indicate a tendency for these slow responders to contribute more paternal care, at least in terms of remaining

close to the nest when a potential intruder is close by. Previous work has shown no relationship between male color and offspring provisioning rate (Maguire and Safran 2010), and no relationship between tail streamer length and incubation (Smith and Montgomerie 1992). However, dominance hierarchies are likely extremely dynamic and complex within barn swallow colonies. Thus, how phenotypic traits translate to social behavior have never been considered in this system, and the underlying reason for the observed relationship between longer latencies in the fast treatment and fledging success remains unknown. Our experimental design represents a snapshot assessment of responses, and future work involving season-long observations of behaviors could provide key insight into this relationship.

Interestingly, and in keeping with previous findings in this population (Chapter 3), predictors of apparent fledging success did not relate to genetic paternity. That is, males responding more slowly to "fast" than "normal" intruders did not have higher paternity in their nests. This result indicates that the processes mediating competition, fledging success, and genetic paternity operate somewhat independently in this system. A previous study (Chapter 3) demonstrated that different aspects of the barn swallow communication system likely evolved through intra- versus intersexual competition, with little overlap in the underlying signals. Specifically, the one axis of color (breast and belly hue), which was shown to predict the spatial separation of competing males, was the only axis of color not shown to be important in female choice (Chapter 3). Yet, here we found an interactive effect of difference in breast brightness on the level of response across rattle tempo

treatments. In our experiment, there was no relationship between the difference in resident and intruder hue and response across rattle tempo treatments. Thus, breast hue may be an important long distance color signal for competing males, while breast brightness is utilized for close-range assessment by both competing males and choosy females. Although chromatic color (i.e. hue) has been shown to be a more reliable signal for discriminating from a distance, in variable light (Schaefer 2006; Cazetta et al. 2009), we know little about how different aspects of color are utilized by receivers at different spatial scales. Thus, the possibility that rattle tempo may reinforce color signals utilized at both of these scales, remains to be tested.

5.6 Conclusion

In this study, resident males' own trill rates did not relate to the strength of response to simulated territorial intrusions. Moreover, contrary to our predictions, there was no consistent increased aggression to fast-trilling intruders, except when the intruder was darker than the resident. This result is consistent with previous studies demonstrating that individuals' quality, relative to competitors, may be more important in determining behavioral responses than absolute measures of competitor signals. However, our findings highlight that signal interactions may be important, and in particular, dynamic signals may need to be backed up by static signals of quality to have an effect on receiver behavior. Within the North American barn swallow, color is uncorrelated with trill rate (Chapter 3), yet we here demonstrate that males only responded more quickly to a faster trilling intruder when the intruder had a darker breast. Thus, because females have been shown to

prefer mates with darker ventral plumage and faster rattles (Chapter 3), and both of these traits are important for mediating male-male competition, it is clear that sexual selection could explain the observed phenotypic divergence in this subspecies. This study therefore highlights the importance of considering signal interactions within and across modalities in studies of the evolution of animal communication systems.

CHAPTER 6

SUMMARY AND CONCLUSIONS

6.1 Summary of key findings

Although long known to be important in phenotypic evolution within populations (Darwin 1871), recent decades have shown increasing interest in the role of sexual selection in speciation (Panhuis et al. 2001; Ritchie 2007; Kraaijeveld et al. 2011; Seddon et al. 2013). This dissertation offers novel insight into both of these processes through a combination of correlational and experimental studies at local and global scales. Within a population of the North American barn swallow (*H. r. erythrogaster*), I provided evidence for the importance of trill rate (rattle tempo) in mediating male-male competition (Chapters 3 and 5), and also demonstrated the importance of this trait in female choice (Chapter 3). I further showed that dark, sexually selected feather plumage is necessary to reinforce trill rate in male territorial interactions (Chapter 5). These findings add to a growing literature on the importance of trills (as high performance traits) in mediating competition (Galeotti et al. 1997; Illes et al. 2006; Schmidt et al. 2008; de Kort et al. 2009; Moseley et al. 2013) and mate choice (Ballentine 2004; Byers et al. 2010b), and also underline the importance of considering and testing receiver perception in studies of acoustic divergence (Seddon and Tobias 2010; Ord 2012). The fact that trill rate was also shown to be important in distinguishing the songs of different barn swallow subspecies (Chapter 4),

highlights that sexual selection processes within populations have implications for population divergence and the potential for speciation.

In Chapter 2, I aimed to generalize beyond my primary study system to synthesize research at the intersection of acoustic divergence and speciation, bringing together studies from arthropods, fish, birds, and mammals. In doing so, I highlighted several gaps in our understanding of the processes shaping acoustic signals; principally, that the relative roles of sexual selection, ecological selection, and drift in driving signal divergence are not generally known for the same species. The closest exception I am aware of is treehoppers, *Enchenopa binotata* (Rodríguez et al. 2006; McNett and Cocroft 2008; Sullivan-Beckers and Cocroft 2010); however, the role of drift in signal divergence has not been considered directly within this species complex. Moreover, I showed that we currently know little about whether signal divergence has a greater role early in the speciation process (in the initiation of pre-zygotic reproductive barriers) or later (in the strengthening of reproductive barriers through character displacement). Additionally, I offered guidelines for future research in this area in the form of a framework of testable hypotheses to differentiate different drivers of acoustic divergence, the acoustic window conceptual framework for understanding how different forms of selection and drift interact with evolutionary constraint to affect signal distributions, and a supplementary table (table 8A2.2) summarizing our current state of knowledge on all these factors across 18 model taxa for acoustic research.

6.2 Contributions to understanding evolutionary processes in the barn swallow study system

The barn swallow *Hirundo rustica* species complex is made up of six closely related yet phenotypically divergent populations. Whereas morphological traits have been quantified among these populations, in Chapter 4, I provided the first description of geographic variation in barn swallow song. This fills an important gap in the barn swallow literature, as a growing number of studies demonstrate divergence in the targets of sexual selection across populations, while song has only been considered for a small fraction of hundreds of papers describing sexual selection in this species, and only within the European subspecies *H. r. rustica* (Møller 1991; Galeotti et al. 1997; Møller et al. 1998; Galeotti et al. 2001; Saino et al. 2003; Garamszegi et al. 2005; Garamszegi et al. 2006a; Dreiss et al. 2008). The finding that barn swallows mostly share a common song structure across their Holarctic distribution provides the foundation for interesting comparative studies on how neurological constraints affect the evolution of learned signals. Specifically, the presence of a warble followed by a rattle in all sampled populations indicates a barn swallow species universal, similar to syntactical constraints shown in several other bird species (Marler 1997). Exceptions to nominate *H. r. rustica* syntax, such as the absence of the p-syllable within *H. r. tytleri*, *H. r. transitiva*, and *H. r. gutturalis*, and the presence of a terminal syllable following the rattle in a few populations, point to interesting song components for future playback studies to assess the function of these song

components in communication. Such studies, in combination with fine-scale population genetic structure, now available through next-generation sequencing, could improve our understanding of how factors such as cultural drift, divergent sexual selection, and syntactical constraints interact in the evolution of birdsong.

Although *H. r. erythrogaste*r song has previously been described qualitatively (Brown 1985), Chapter 3 represents the first quantitative characterization of song for this subspecies, which is quickly becoming an important comparative model for research in sexual selection and speciation (Safran and McGraw 2004; Safran et al. 2005; Kleven et al. 2006; Neuman et al. 2007; Safran et al. 2008; Dor et al. 2010; Safran et al. 2010; Eikenaar et al. 2011; Lifjeld et al. 2011; Safran et al. 2012; Vitousek et al. 2013). Moreover, of the myriad papers on barn swallow sexual selection, only one other paper I am aware of has directly considered the function of signaling traits (in this case, the rattle) in intrasexual selection (Galeotti et al. 1997). Chapter 3 is therefore the first paper to consider the relative roles of intra- and intersexual selection in the evolution of the barn swallow multimodal communication system. It may also be the first study to do so for any system, as the role of signals in intra- and intersexual selection have typically been considered for a single trait within a single modality for taxa in which it has been studied (Jones and Hunter 1999; Hoi and Griggio 2008; Sullivan-Beckers and Cocroft 2010; Dennenmoser and Christy 2013). My dissertation work therefore represents an important first step in characterizing the likely selection pressures affecting different aspects of

multimodal phenotypes which can be leveraged for future comparative, experimental, and quantitative genomic studies.

6.3 Implications for the study of signal divergence

In Chapter 2, I proposed the acoustic window concept (Section 2.4 and Figure 2.1) as a new way to incorporate selection, drift, available acoustic space, and physiological constraints into a unified framework. This framework subsumes the acoustic adaptation (Morton 1975), sensory bias (Ryan and Rand 1993a), and sensory drive (Endler 1992) hypotheses, and provides greater conceptual focus on the direct mechanisms of evolutionary change. Additionally, this framework is conducive to modeling adaptation in multidimensional parameter space (to incorporate multiple axes of variation, such as frequency, temporal characteristics, or complexity metrics), and could easily be adapted to other modalities, such as visual or electrical signaling.

In Chapter 2, I also provided a table (2.2) with testable hypotheses for determining the primary drivers of acoustic divergence. By providing general expectations, as well as predictions related to different experimental and correlational studies, I hope this set of hypotheses stimulates researchers to fill gaps in our knowledge of how different selective regimes lead to signal divergence across broad taxonomic groups. Additionally, I proposed a set of predictions for determining the timeframe over which acoustic divergence plays a role in population divergence (figure 2.2). If these predictions are tested across a variety of taxa, this would greatly improve our understanding of how changes

in signals and communication systems function in the process of speciation. Collectively, I hope the synthesis and conceptual framework provided in Chapter 2 will afford researchers with new perspective on gaps in our knowledge and appropriate methods for addressing them across taxa and will greatly further research in the area of acoustics and speciation.

6.4 Implications for the study of complex signal evolution

In Chapter 3, I proposed a novel approach for visualizing complex signaling systems using correlation-based phenotype networks and mapping on trait functions following model selection. By further proposing clustering arrangements predicted by robust over-design as a null model, I then revealed a constraining factor of male natural history (the hazards of agonistic encounters) which likely prevent signals mediating male-male competition from obtaining this clustering pattern. As visualizing complex signal interactions has been indicated as a major challenge for research (Partan 2013), I hope this approach will afford researchers new perspectives on their data to spark new experiments and theory. Furthermore, as robust over-design has been proposed as a general organizing principle of biological systems (Krakauer and Plotkin 2005; Ay et al. 2007), using this as a null hypothesis for testing the phenotype networks associated with diverse taxa and signaling contexts should provide novel perspectives on sender-receiver dynamics and life history constraints. A prime application of this framework would be to flower-pollinator signaling systems,

which utilize a multitude of visual, chemical, and olfactory traits to interact with various pollinator guilds.

6.5 Future directions

The phenotype network approach for studying complex signaling has many obvious extensions in our study system. I have already developed a preliminary network for female barn swallow multimodal signaling, including song, which has thus far been considered a copulation solicitation call in the literature (del Hoyo and Elliott 2014). However, recent studies have indicated that singing females represent the ancestral state of Northern Hemisphere passerines (Garamszegi et al. 2006b; Price et al. 2009) and that cryptic female song exists under particular behavioral conditions (Taff et al. 2012). I therefore aim to utilize the methodological approach in Chapter 3 to compare phenotype networks for male and female barn swallows in a forthcoming paper.

In addition, I have an ongoing collaboration (since 2009) with Dr. Hakan Karaardıç, of Akdeniz University, Turkey, to study sexual selection pressures within a phenotypically diverse population of barn swallows in southwestern Turkey. Although this population is genetically similar to other mainland European *H. r. rustica* populations, it exhibits broad variation in ventral color and tail streamer length, which may indicate gene flow with the eastern Mediterranean subspecies *H. r. transitiva.* We are currently writing a manuscript characterizing the phenotypic traits associated with apparent versus genetic reproductive success within this population.

Another project I am currently working on with Dr. Clint Francis, of California Polytechnic State University, involves application of metabolic theory of ecology (MTE) to understand the processes affecting song evolution across birds. Because the effects of body mass on metabolic limits predicts overall signal pitch and duration across the major acoustically signaling animal lineages (Gillooly and Ophir 2010), we aim to utilize these as baseline predictions for signal characteristics for 795 bird species. By testing whether degree of dichromatism or median latitude for these species better predicts divergence from MTE predictions, we will be able to infer effects of selection from a null distribution.

6.6 Conclusions

The study of complex signal evolution has become a rapidly growing research area in recent decades, as has the study of the role of sexual selection in speciation. My dissertation research provides novel insight into the differential roles of intra- and intersexual selection in shaping aspects of the barn swallow communication system within and across populations. I additionally provide experimental evidence demonstrating the function of a divergent song trait in mediating fine-scale agonistic interactions, with implications for the importance of static signals in reinforcing uncorrelated dynamic signals. I hope that my empirical work on multimodal sexual signaling within barn swallows, and my synthesis of the causes and consequences of acoustic divergence across animal

taxa provide the foundation for much future work at the interface of sexual selection, signal evolution, and speciation.

7. REFERENCES

Akre, K. L., H. E. Farris, A. M. Lea, R. A. Page, and M. J. Ryan. 2011. Signal Perception in Frogs and Bats and the Evolution of Mating Signals. Science 333:751–752.

Amézquita, A., S. V. Flechas, A. P. Lima, H. Gasser, and W. Hödl. 2011. Acoustic interference and recognition space within a complex assemblage of dendrobatid frogs. Proceedings of the National Academy of Sciences of the United States of America 108:17058–63.

Amézquita, A., A. P. Lima, R. Jehle, L. Castellanos, Ó. Ramos, A. J. Crawford, H. Gasser, et al. 2009. Calls, colours, shape, and genes: a multi-trait approach to the study of geographic variation in the Amazonian frog Allobates femoralis. Biological Journal of the Linnean Society 98:826–838.

Amorim, M. C. P., J. M. Simões, P. J. Fonseca, and G. F. Turner. 2008. Species differences in courtship acoustic signals among five Lake Malawi cichlid species (Pseudotropheus spp.). Journal of Fish Biology 72:1355–1368.

Amrhein, V., and N. Erne. 2006. Dawn singing reflects past territorial challenges in the winter wren. Animal Behaviour 71:1075–1080.

Anderson, D., K. Burnham, and W. Thompson. 2000. Null hypothesis testing: problems, prevalence, and an alternative. The Journal of Wildlife Management 64:912–923.

Andersson, M. B. 1994. Sexual Selection. Princeton University Press.

Andersson, M., and L. W. Simmons. 2006. Sexual selection and mate choice. Trends in ecology & evolution (Personal edition) 21:296–302.

Andersson, S., S. R. Pryke, J. Ornborg, M. J. Lawes, and M. Andersson. 2002. Multiple receivers, multiple ornaments, and a trade-off between agonistic and epigamic signaling in a widowbird. The American Naturalist 160:683–91.

Arnegard, M. E., P. B. McIntyre, L. J. Harmon, M. L. Zelditch, W. G. R. Crampton, J. K. Davis, J. P. Sullivan, et al. 2010. Sexual Signal Evolution Outpaces Ecological Divergence during Electric Fish Species Radiation. The American Naturalist 176:335–56.

Ay, N., J. Flack, and D. C. Krakauer. 2007. Robustness and complexity co-constructed in multimodal signalling networks. Philosophical transactions of the Royal Society of London. Series B, Biological sciences 362:441–7.

Baker, M. C., T. K. Bjerke, H. Lampe, and Y. Espmark. 1986. Sexual Response of Female Great Tits to Variation in Size of Males' Song Repertoires. The American Naturalist 128:491–498.

Balakrishnan, C. N., K. M. Sefc, and M. D. Sorenson. 2009. Incomplete reproductive isolation following host shift in brood parasitic indigobirds. Proceedings of the Royal Society B: Biological Sciences 276:219–28.

Ballentine, B. 2004. Vocal performance influences female response to male bird song: an experimental test. Behavioral Ecology 15:163–168.

Baptista, L. F., and P. W. Trail. 1992. The role of song in the evolution of passerine diversity. Systematic Biology 41:242–247.

Barton, K. 2014. MuMIn: Multi-model inference.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2014. lme4: Linear mixed-effects models using Eigen and S4.

Beecher, M. D., and E. a Brenowitz. 2005. Functional aspects of song learning in songbirds. Trends in Ecology & Evolution 20:143–9.

Bensch, S., T. Price, J. Kohn, and others. 1997. Isolation and characterization of microsatellite loci in a Phylloscopus warbler. Molecular Ecology 6:91–92.

Boncoraglio, G., and N. Saino. 2007. Habitat structure and the evolution of bird song: a meta-analysis of the evidence for the acoustic adaptation hypothesis. Functional Ecology 21:134–142.

Bonduriansky, R. 2011. Sexual selection and conflict as engines of ecological diversification. The American Naturalist 178:729–45.

Bosch, J., A. Rand, and M. Ryan. 2000. Signal variation and call preferences for whine frequency in the túngara frog, Physalaemus pustulosus. Behavioral Ecology and Sociobiology 49:62–66.

Boughman, J. W. 2001. Divergent sexual selection enhances reproductive isolation in sticklebacks. Nature 411:944–8.

Boul, K. E., W. C. Funk, C. R. Darst, D. C. Cannatella, and M. J. Ryan. 2007. Sexual selection drives speciation in an Amazonian frog. Proceedings of the Royal Society B: Biological Sciences 274:399–406.

Bowman, R. I. 1979. Adaptive morphology of song dialects in Darwin's finches. Journal fur Ornithologie 120:353–389.

Braune, P., S. Schmidt, and E. Zimmermann. 2008. Acoustic divergence in the communication of cryptic species of nocturnal primates (Microcebus ssp.). BMC Biology 6:19.

Briefer, E., E. Vannoni, and A. G. McElligott. 2010. Quality prevails over identity in the sexually selected vocalisations of an ageing mammal. BMC Biology 8:1–15.

Bro-Jørgensen, J. 2010. Dynamics of multiple signalling systems: animal communication in a world in flux. Trends in Ecology & Evolution 25:292–300.

Brown, C. R. 1985. Vocalizations of Barn and Cliff Swallows. The Southwestern Naturalist 30:325–333.

Brown, C. R. 1996. Coloniality in the cliff swallow: the effect of group size on social behavior. University of Chicago Press.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multi-model inference: a practical information-theoretic approach. Springer.

Burnham, K. P., D. R. Anderson, and K. P. Huyvaert. 2010. AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons. Behavioral Ecology and Sociobiology 65:23–35.

Byers, B. E., K. L. Belinsky, and R. A. Bentley. 2010a. Independent cultural evolution of two song traditions in the chestnut-sided warbler. The American Naturalist 176:476–89.

Byers, B. E., and D. E. Kroodsma. 2009. Female mate choice and songbird song repertoires. Animal Behaviour 77:13–22.

Byers, J., E. Hebets, and J. Podos. 2010b. Female mate choice based upon male motor performance. Animal Behaviour 79:771–778.

Cade, W., and E. Cade. 1992. Male mating success, calling and searching behaviour at high and low densities in the field cricket, Gryllus integer. Animal Behaviour 43:49–56.

Cade, W. H. 1991. Inter- and intraspecific variation in nightly calling duration in field crickets,Gryllus integer andG. rubens (Orthoptera: Gryllidae). Journal of Insect Behavior 4:185–194.

Cade, W. H., and D. Otte. 2000. Gryllus texensis n. sp.: A Widely Studied Field Cricket (Orthoptera; Gryllidae) from the Southern United States. Transactions of the American Entomological Society 126:117–123.

Cade, W. H., and M. G. Tyshenko. 1990. Geographic variation in hybrid fertility in the field crickets Gryllus integer, G. rubens, and Gryllus sp. Canadian Journal of Zoology 68:2697–2700.

Campbell, D. L. M., and M. E. Hauber. 2009. Cross-fostering diminishes song discrimination in zebra finches (Taeniopygia guttata). Animal cognition 12:481–90.

Campbell, P., B. Pasch, J. L. Pino, O. L. Crino, M. Phillips, and S. M. Phelps. 2010. Geographic variation in the songs of Neotropical singing mice: testing the relative importance of drift and local adaptation. Evolution 64:1955–72.

Candolin, U. 2003. The use of multiple cues in mate choice. Biological Reviews of the Cambridge Philosophical Society 78:575–95.

Cap, H., P. Deleporte, J. Joachim, and D. Reby. 2008. Male vocal behavior and phylogeny in deer. Cladistics 24:917–931.

Capranica, R., and L. Frishkopf. 1973. Encoding of geographic dialects in the auditory system of the cricket frog. Science 182:1272–4.

Cardoso, G. C., and Y. Hu. 2011. Birdsong performance and the evolution of simple (rather than elaborate) sexual signals. The American Naturalist 178:679–686.

Catchpole, C. K. 1987. Bird song, sexual selection and female choice. Trends in Ecology & Evolution 2:94–7.

Catchpole, C. K., and P. J. B. Slater. 2003. Bird song: biological themes and variations (p. 10). Cambridge University Press.

Cazetta, E., H. M. Schaefer, and M. Galetti. 2009. Why are fruits colorful? The relative importance of achromatic and chromatic contrasts for detection by birds. Evolutionary Ecology 23:233–244.

Charlton, B. D., D. Reby, and K. McComb. 2007. Female red deer prefer the roars of larger males. Biology letters 3:382–5.

Chek, A. a., J. P. Bogart, and S. C. Lougheed. 2003. Mating signal partitioning in multi-species assemblages: a null model test using frogs. Ecology Letters 6:235–247.

Claridge, M. F., and J. C. Morgan. 1993. Geographical variation i n acoustic signals of the planthopper , Nilaparvata bakeri (Muir), i n Asia□: species recognition and sexual selection. October 267–281.

Clayton, N. 1990. Subspecies recognition and song learning in zebra finches. Animal Behaviour 40:1009–1017.

Cocroft, R. B., R. L. Rodríguez, and R. E. Hunt. 2008. Host Shifts, the Evolution of Communication, and Speciation in the Enchenopa binotata Species Complex of Treehoppers. The Evolutionary Biology Of Herbivorous Insects: Specialization, Speciation And Radiation (pp. 88–100).

Cocroft, R. B., R. L. Rodríguez, and R. E. Hunt. 2010. Host shifts and signal divergence: mating signals covary with host use in a complex of specialized plant-feeding insects. Biological Journal of the Linnean Society 99:60–72.

Cohen, J. 1988. Statistical power analysis for the behavioral sciencies (2nd Ed.). Erlbaum, Hillsdale, NJ.

Colegrave, N., H. Hollocher, K. Hinton, and M. G. Ritchie. 2000. The courtship song of African Drosophila melanogaster. Journal of Evolutionary Biology 13:143–150.

Collins, S. 2004. Vocal fighting and flirting: the functions of birdsong. In P. Marler & H. Slabbekoorn, eds., Nature's music: the science of birdsong (pp. 39–79). Elsevier Academic Press San Diego, California, USA.

Collins, S. A., S. R. de Kort, J. Pérez-Tris, and J. L. Tellería. 2009. Migration strategy and divergent sexual selection on bird song. Proceedings of the Royal Society B: Biological Sciences 276:585–90.

Dalziell, A. H., and A. Cockburn. 2008. Dawn song in superb fairy-wrens: a bird that seeks extrapair copulations during the dawn chorus. Animal Behaviour 75:489–500.

Danchin, E., L.-A. Giraldeau, T. J. Valone, and R. H. Wagner. 2004. Public information: from nosy neighbors to cultural evolution. Science 305:487–91.

Darwin, C. 1871. The Descent of Man and Selection in Relation to Sex. Murray, London.

De Kort, S. R., E. R. B. Eldermire, S. Valderrama, C. a Botero, and S. L. Vehrencamp. 2009. Trill consistency is an age-related assessment signal in banded wrens. Proceedings of the Royal Society B: Biological Sciences 276:2315–21.

Del Hoyo, J., and A. Elliott, eds. 2014. Barn Swallow (Hirundo rustica). Handbook of the Birds of the World: Alive. Lynx.

Delgado, R. a. 2007. Geographic Variation in the Long Calls of Male Orangutans (Pongo spp.). Ethology 113:487–498.

Dennenmoser, S., and J. H. Christy. 2013. The design of a beautiful weapon: compensation for opposing sexual selection on a trait with two functions. Evolution; international journal of organic evolution 67:1181–8.

Derryberry, E. P., N. Seddon, S. Claramunt, J. A. Tobias, A. Baker, A. Aleixo, and R. T. Brumfield. 2012. Correlated evolution of beak morphology and song in the neotropical woodcreeper radiation. Evolution 66:2784–97.

DeVoogd, T. J. 2004. Neural constraints on the complexity of avian song. Brain, Behavior and Evolution 63:221–32.

Dor, R., R. J. Safran, F. H. Sheldon, D. W. Winkler, and I. J. Lovette. 2010. Phylogeny of the genus Hirundo and the Barn Swallow subspecies complex. Molecular phylogenetics and evolution 56:409–18.

Drăgănoiu, T. I., L. Nagle, and M. Kreutzer. 2002. Directional female preference for an exaggerated male trait in canary (Serinus canaria) song. Proceedings of the Royal Society B: Biological Sciences 269:2525–31.

Dray, S., A.-B. Dufour, and others. 2007. The ade4 package: implementing the duality diagram for ecologists. Journal of statistical software 22:1–20.

Dreiss, A., C. Navarro, F. De Lope, and A. Møller. 2008. Effects of an immune challenge on multiple components of song display in barn swallows Hirundo rustica: implications for sexual selection. Ethology 114:955–964.

Eikenaar, C., M. Whitham, J. Komdeur, M. van der Velde, and I. T. Moore. 2011. Testosterone, Plumage Colouration and Extra-Pair Paternity in Male North-American Barn Swallows. PLoS ONE 6:e23288.

Elias, D. O., A. C. Mason, and E. A. Hebets. 2010. A signal-substrate match in the substrate-borne component of a multimodal courtship display. Current Zoology 56:370–378.

Endler, J. A. 1992. Signals, Signal Conditions, and the Direction of Evolution. The American Naturalist 139:S125–S153.

Endler, J., and A. Basolo. 1998. Sensory ecology, receiver biases and sexual selection. Trends in Ecology & Evolution 5347:415–420.

Epskamp, S., A. O. J. Cramer, L. J. Waldorp, V. D. Schmittmann, and D. Borsboom. 2012. qgraph□: Network Visualizations of Relationships in Psychometric Data. Journal of Statistical Software 48:1–18.

Ferrer-i-Cancho, R., A. Hernández-Fernández, D. Lusseau, G. Agoramoorthy, M. J. Hsu, and S. Semple. 2013. Compression as a universal principle of animal behavior. Cognitive science 37:1565–78.

Filatova, O. a., V. B. Deecke, J. K. B. Ford, C. O. Matkin, L. G. Barrett-Lennard, M. a. Guzeev, A. M. Burdin, et al. 2012. Call diversity in the North Pacific killer whale populations: implications for dialect evolution and population history. Animal Behaviour 83:595–603.

Fitzpatrick, M. J., and D. a. Gray. 2001. Divergence between the Courtship Songs of the Field Crickets Gryllus texensis and Gryllus rubens (Orthoptera, Gryllidae). Ethology 107:1075–1085.

Forstmeier, W., C. Burger, K. Temnow, and S. Derégnaucourt. 2009. The genetic basis of zebra finch vocalizations. Evolution; international journal of organic evolution 63:2114–30.

Francis, C. D., C. P. Ortega, and A. Cruz. 2011. Noise Pollution Filters Bird Communities Based on Vocal Frequency. (S. A. Rands, ed.)PLoS ONE 6:e27052.

Fruchterman, T. M. J., and E. M. Reingold. 1991. Graph drawing by force-directed placement. Software: Practice and Experience 21:1129–1164.

Fujita, G., and H. Higuchi. 2006. Barn swallows prefer to nest at sites hidden from neighboring nests within a loose colony. Journal of Ethology 25:117–123.

Funk, W. C., A. Angulo, J. P. Caldwell, M. J. Ryan, and D. C. Cannatella. 2008. Comparison of Morphology and Calls of Two Cryptic Species of Physalaemus (Anura: Leiuperidae). Herpetologica 64:290–304.

Funk, W. C., M. Caminer, and S. R. Ron. 2012. High levels of cryptic species diversity uncovered in Amazonian frogs. Proceedings of the Royal Society B: Biological Sciences 279:1806–1814.

Funk, W. C., D. C. Cannatella, and M. J. Ryan. 2009. Genetic divergence is more tightly related to call variation than landscape features in the Amazonian frogs Physalaemus petersi and P. freibergi. Journal of Evolutionary Biology 22:1839–53.

Galeotti, P., N. Saino, E. Perani, R. Sacci, and A. P. Møller. 2001. Age-related song variation in male barn swallows. Italian Journal of Zoology 68:305–310.

Galeotti, P., N. Saino, R. Sacchi, and A. P. Møller. 1997. Song correlates with social context, testosterone and body condition in male barn swallows. Animal Behaviour 53:687–700.

Gamble, T., P. B. Berendzen, H. Bradley Shaffer, D. E. Starkey, and A. M. Simons. 2008. Species limits and phylogeography of North American cricket frogs (Acris: Hylidae). Molecular phylogenetics and evolution 48:112–25.

Garamszegi, L. Z. 2010. Information-theoretic approaches to statistical analysis in behavioural ecology: an introduction. Behavioral Ecology and Sociobiology 65:1–11.

Garamszegi, L. Z., G. Hegyi, D. Heylen, P. Ninni, F. de Lope, M. Eens, and A. P. Møller. 2006a. The design of complex sexual traits in male barn swallows: associations between signal attributes. Journal of Evolutionary Biology 19:2052–66.

Garamszegi, L. Z., D. Heylen, A. P. Møller, M. Eens, and F. De Lope. 2005. Age-dependent health status and song characteristics in the barn swallow. Behavioral Ecology 16:580–591.

Garamszegi, L. Z., D. Z. Pavlova, M. Eens, and a. P. Moller. 2006b. The evolution of song in female birds in Europe. Behavioral Ecology 18:86–96.

Gee, J. M. 2005. No species barrier by call in an avian hybrid zone between California and Gambel's quail (Callipepla californica and C . gambelii). Biological Journal of the Linnean Society 86:253–264.

Gil, D., and P. J. B. Slater. 2000. Multiple song repertoire characteristics in the willow warbler (Phylloscopus trochilus): correlations with female choice and offspring viability. Behavioral Ecology and Sociobiology 47:319–326.

Gillooly, J. F., and A. G. Ophir. 2010. The energetic basis of acoustic communication. Proceedings. Biological Sciences / The Royal Society 277:1325–31.

Girard, M. B., M. M. Kasumovic, and D. O. Elias. 2011. Multi-modal courtship in the peacock spider, Maratus volans (O.P.-Cambridge, 1874). PloS ONE 6:e25390.

Gottlander, K. 1987. Variation in the song rate of the male pied flycatcher Ficedula hypoleuca: causes and consequences. Animal Behaviour 35:1037–1043.

Grace, J. L., and K. L. Shaw. 2011. Coevolution of male mating signal and female preference during early lineage divergence of the Hawaiian cricket, Laupala cerasina. Evolution 65:2184–96.

Grace, J. L., and K. L. Shaw. 2012. Incipient sexual isolation in Laupala cerasina: Females discriminate population-level divergence in acoustic characters. Current Zoology (in press).

Grant, B. R., and P. R. Grant. 1996. Cultural Inheritance of Song and Its Role in the Evolution of Darwin's Finches. Evolution 50:2471–2487.

Grant, B. R., and P. R. Grant. 2010. Songs of Darwin's finches diverge when a new species enters the community. Proceedings of the National Academy of Sciences of the United States of America 107:20156–63.

Gray, D. A., and W. H. Cade. 1999. Quantitative Genetics of Sexual Selection in the Field Cricket, Gryllus integer. Evolution 53:848–854.

Gray, D. A., and W. H. Cade. 2000. Sexual selection and speciation in field crickets. Proceedings of the National Academy of Sciences of the United States of America 97:14449–54.

Gray, D. A., and G. Eckhardt. 2001. Is cricket courtship song condition dependent? Animal Behaviour 62:871–877.

Grether, G. F., N. Losin, C. N. Anderson, and K. Okamoto. 2009. The role of interspecific interference competition in character displacement and the evolution of competitor recognition. Biological reviews of the Cambridge Philosophical Society 84:617–35.

Grueber, C. E., S. Nakagawa, R. J. Laws, and I. G. Jamieson. 2011. Multimodel inference in ecology and evolution: challenges and solutions. Journal of Evolutionary Biology 24:699–711.

Guerra, M. a., and S. R. Ron. 2008a. Mate choice and courtship signal differentiation promotes speciation in an Amazonian frog. Behavioral Ecology 19:1128–1135.

Guerra, M. A., and S. R. Ron. 2008b. Mate choice and courtship signal differentiation promotes speciation in an Amazonian frog. Behavioral Ecology 19:1128–1135.

Guilford, T., and M. S. Dawkins. 1993. Receiver psychology and the design of animal signals. Trends in Neurosciences 16:430–6.

Guilford, T. I. M., and M. S. Dawkins. 1991. Receiver psychology and the evolution of animal signals. Animal Behaviour 42:1–14.

Haavie, J., T. Borge, S. Bures, L. Z. Garamszegi, H. M. Lampe, J. Moreno, A. Qvarnström, et al. 2004. Flycatcher song in allopatry and sympatry - convergence, divergence and reinforcement. Journal of Evolutionary Biology 17:227–237.

Hack, M. A. 1998. The Energetics of Male Mating Strategies in Field Crickets (Orthoptera: Gryllinae: Gryllidae). Journal of Insect Behavior 11:853–867.

Hanotte, O., C. Zanon, A. Pugh, C. Greig, A. Dixon, and T. Burke. 1994. Isolation and characterization of microsatellite loci in a passerine bird: the reed bunting Emberiza schoeniclus. Molecular Ecology 3:529–530.

Hasegawa, M., E. Arai, M. Watanabe, and M. Nakamura. 2010. Mating advantage of multiple male ornaments in the Barn Swallow Hirundo rustica gutturalis. Ornithological Science 9:141–148.

Hebets, E. A. 2011. Current status and future directions of research in complex signaling. Current Zoology 57:i–v.

Hebets, E. A., and D. R. Papaj. 2005. Complex signal function: developing a framework of testable hypotheses. Behavioral Ecology and Sociobiology 57:197–214.

Hebets, E. A., C. J. Vink, L. Sullivan-Beckers, and M. F. Rosenthal. 2013. The dominance of seismic signaling and selection for signal complexity in Schizocosa multimodal courtship displays. Behavioral Ecology and Sociobiology 67:1483–1498.

Hedrick, A. V. 1986. Female preferences for male calling bout duration in a field cricket. Behavioral Ecology and Sociobiology 19:73–77.

Hedrick, A. V. 1988. Female Choice and the Heritability of Attractive Male Traits: An Empirical Study. The American Naturalist 132:267–276.

Hendry, A. P., S. K. Huber, L. F. De León, A. Herrel, and J. Podos. 2009. Disruptive selection in a bimodal population of Darwin's finches. Proceedings of the Royal Society B: Biological Sciences 276:753–9.

Henry, C. 1994. Singing and cryptic speciation in insects. Trends in Ecology & Evolution 9:388–392.

Henry, C. S. 1985a. The Proliferation of Cryptic Species in Chrysoperla Green Lacewings through Song Divergence. The Florida Entomologist 68:18–38.

Henry, C. S. 1985b. Sibling Species, Call Differences, and Speciation in Green Lacewings (Neuroptera: Chrysopidae: chrysoperla). Evolution 39:965–984.

Henry, C. S., M. L. Martínez Wells, and K. E. Holsinger. 2002. The inheritance of mating songs in two cryptic, sibling lacewing species (Neuroptera: Chrysopidae: Chrysoperla). Genetica 116:269–89.

Henry, C. S., and M. L. M. Wells. 2004. Adaptation or random change? The evolutionary response of songs to substrate properties in lacewings (Neuroptera: Chrysopidae: Chrysoperla). Animal Behaviour 68:879–895.

Henry, C. S., and M. L. M. Wells. 2006. Testing the ability of males and females to respond to altered songs in the dueting green lacewing, Chrysoperla plorabunda (Neuroptera: Chrysopidae). Behavioral Ecology and Sociobiology 61:39–51.

Henry, C. S., and M. L. M. Wells. 2009. Sexually Dimorphic Intrasexual Duetting in an Otherwise Monomorphic Green Lacewing (Neuroptera, Chrysopidae, Chrysoperla plorabunda): Sexual Selection or Sex Recognition? Journal of Insect Behavior 22:289–312.

Henry, C. S., M. L. M. Wells, and C. M. Simon. 1999. Convergent Evolution of Courtship Songs among Cryptic Species of the Carnea Group of Green Lacewings (Neuroptera: Chrysopidae: Chrysoperla). Evolution 53:1165–1179.

Henry, C. S., and M. M. Wells. 1990. Geographical variation in the song of Chrysoperla plorabunda (Neuroptera: Chrysopidae) in North America. Annals of the Entomological Society of America 83:317–325.

Higashi, M., G. Takimoto, and N. Yamamura. 1999. Sympatric speciation by sexual selection. Nature 402:523–6.

Higgins, L., and R. Waugaman. 2004. Sexual selection and variation: a multivariate approach to species-specific calls and preferences. Animal Behaviour 68:1139–1153.

Higham, J. P., and E. A. Hebets. 2013. An introduction to multimodal communication. Behavioral Ecology and Sociobiology 67:1381–1388.

Hoback, W. W., and J. Wagner, William E. 1997. The energetic cost of calling in the variable field cricket, Gryllus lineaticeps. Physiological Entomology 22:286–290.

Hoi, H., and M. Griggio. 2008. Dual Utility of a Melanin-Based Ornament in Bearded Tits. Ethology 114:1094–1100.

Hoskin, C. J., and M. Higgie. 2010. Speciation via species interactions: the divergence of mating traits within species. Ecology Letters 13:409–20.

Hoskin, C. J., M. Higgie, K. R. McDonald, and C. Moritz. 2005. Reinforcement drives rapid allopatric speciation. Nature 437:1353–6.

Houtman, a. M. 1992. Female Zebra Finches Choose Extra-Pair Copulations with Genetically Attractive Males. Proceedings of the Royal Society B: Biological Sciences 249:3–6.

Huber, S. K., and J. Podos. 2006. Beak morphology and song features covary in a population of Darwin's finches (Geospiza fortis). Biological Journal of the Linnean Society 88:489–498.

Hunt, J., C. J. Breuker, J. A. Sadowski, and A. J. Moore. 2009. Male-male competition, female mate choice and their interaction: determining total sexual selection. Journal of evolutionary biology 22:13–26.

Hunter, M. L., and J. R. Krebs. 1979. Geographical Variation in the Song of the Great Tit (Parus major) in Relation to Ecological Factors. The Journal of Animal Ecology 48:759.

Hurlbert, S. H. 1984. Pseudoreplication and the Design of Ecological Field Experiments. Ecological Monographs 54:187.

Illes, A. E., M. L. Hall, and S. L. Vehrencamp. 2006. Vocal performance influences male receiver response in the banded wren. Proceedings. Biological sciences / The Royal Society 273:1907–12.

Irwin, D. E. 2000. Song Variation in an Avian Ring Species. Evolution 54:998–1010.

Irwin, D. E. 2012. Culture in songbirds and its contribution to the evolution of new species. In E. Slingerland & M. Collard, eds., Creating Consilience: Integrating the Sciences and the Humanities (pp. 163–178). Oxford Univ Press, Oxford.

Irwin, D. E., P. Alström, U. Olsson, and Z. M. Benowitz-Fredericks. 2001a. Cryptic species in the genus Phylloscopus (Old World leaf warblers). Ibis 143:233–247.

Irwin, D. E., S. Bensch, and T. D. Price. 2001b. Speciation in a ring. Nature 409:333–7.

Irwin, D. E., M. P. Thimgan, and J. H. Irwin. 2008. Call divergence is correlated with geographic and genetic distance in greenish warblers (Phylloscopus trochiloides): a strong role for stochasticity in signal evolution? Journal of Evolutionary Biology 21:435–48.

Jain, M., and R. Balakrishnan. 2012. Does acoustic adaptation drive vertical stratification? A test in a tropical cricket assemblage. Behavioral Ecology 23:343–354.

Johnstone, R. A. 1995. Honest advertisement of multiple qualities using multiple signals. Journal of Theoretical Biology 177:87–94.

Johnstone, R. A. 1996. Multiple Displays in Animal Communication:`Backup Signals' and `Multiple Messages'. Philosophical Transactions of the Royal Society B: Biological Sciences 351:329–338.

Jones, G. 1997. Acoustic signals and speciation: the roles of natural and sexual selection in the evolution of cryptic species. Advances in the Study of Behaviour 26:317–354.

Jones, G., and B. M. Siemers. 2011. The communicative potential of bat echolocation pulses. Journal of Comparative Physiology A 197:447–57.

Jones, I., and F. Hunter. 1999. Experimental evidence for mutual inter- and intrasexual selection favouring a crested auklet ornament. Animal behaviour 57:521–528.

Jonsson, T., E. A. Kravitz, and R. Heinrich. 2011. Sound production during agonistic behavior of male Drosophila melanogaster. Fly 5:29–38.

Kalinowski, S. T., M. L. Taper, and T. C. Marshall. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology 16:1099–106.

Kime, N. M., W. R. Turner, and M. J. Ryan. 2000. The transmission of advertisement calls in Central American frogs. Behavioral Ecology 11:71–83.

Kingston, T., and S. J. Rossiter. 2004. Harmonic-hopping in Wallacea's bats. Nature 429:654–7.

Kirschel, A. N. G., D. T. Blumstein, R. E. Cohen, W. Buermann, T. B. Smith, and H. Slabbekoorn. 2009a. Birdsong tuned to the environment: green hylia song varies with elevation, tree cover, and noise. Behavioral Ecology 20:1089–1095.

Kirschel, A. N. G., D. T. Blumstein, and T. B. Smith. 2009b. Character displacement of song and morphology in African tinkerbirds. Proceedings of the National Academy of Sciences of the United States of America 106:8256–61.

Kleven, O., F. Jacobsen, and J. T. Lifjeld. 2006. Male tail streamer length predicts fertilization success in the North American barn swallow (Hirundo rustica erythrogaster). Behavioral Ecology and Sociobiology 59:412–418.

Koetz, A. H., D. A. Westcott, and B. C. Congdon. 2007. Geographical variation in song frequency and structure: the effects of vicariant isolation, habitat type and body size. Animal Behaviour 74:1573–1583.

Kojima, W., W. Kitamura, S. Kitajima, Y. Ito, K. Ueda, G. Fujita, and H. Higuchi. 2009. Female Barn Swallows Gain Indirect but not Direct Benefits through Social Mate Choice. Ethology 115:939–947.

Kraaijeveld, K., F. J. L. Kraaijeveld-Smit, and M. E. Maan. 2011. Sexual selection and speciation: the comparative evidence revisited. Biological reviews of the Cambridge Philosophical Society 86:367–77.

Krakauer, D., and J. Plotkin. 2005. Principles and parameters of molecular robustness. In E. Jen, ed., Robust Design: A Repertoire of Biological, Ecological and Engineering Case Studies (pp. 71–103). Oxford University Press.

Kramer, H. G., and R. E. Lemon. 1983. Dynamics of Territorial Singing between Neighboring Song Sparrows (Melospiza melodia). Behaviour 85:198–223.

Kroodsma, D. E., B. E. Byers, E. Goodale, S. Johnson, and W.-C. Liu. 2001. Pseudoreplication in playback experiments, revisited a decade later. Animal Behaviour 61:1029–1033.

Lachlan, R. F., and M. R. Servedio. 2004. Song learning accelerates allopatric speciation. Evolution 58:2049–63.

Lampe, H. M., and Y. O. Espmark. 2002. Mate choice in pied flycatchers Ficedula hypoleuca: can females use song to find high-quality males and territories? Ibis 145:E24–E33.

Lampe, H. M., and G.-P. Saetre. 1995. Female Pied Flycatchers Prefer Males with Larger Song Repertoires. Proceedings of the Royal Society B: Biological Sciences 262:163–167.

Langerhans, R. B. 2010. Predicting evolution with generalized models of divergent selection: a case study with poeciliid fish. Integrative and Comparative Biology 50:1167–84.

Lifjeld, J. T., O. Kleven, F. Jacobsen, K. J. McGraw, R. J. Safran, and R. J. Robertson. 2011. Age before beauty? Relationships between fertilization success and age-dependent ornaments in barn swallows. Behavioral ecology and sociobiology 65:1687–1697.

Liu, I., B. Lohr, B. Olsen, and R. Greenberg. 2008. Macrogeographic vocal variation in subspecies of swamp sparrow. The Condor 110:102–109.

Lukacs, P. M., W. L. Thompson, W. L. Kendall, W. R. Gould, P. F. Doherty, K. P. Burnham, and D. R. Anderson. 2007. Concerns regarding a call for pluralism of information theory and hypothesis testing. Journal of Applied Ecology 44:456–460.

Luther, D. A., and E. P. Derryberry. 2012. Birdsongs keep pace with city life: changes in song over time in an urban songbird affects communication. Animal Behaviour 83:1059–1066.

Maan, M. E., and O. Seehausen. 2011. Ecology, sexual selection and speciation. Ecology Letters 14:591–602.

MacDougall-Shackleton, E. a, and S. a MacDougall-Shackleton. 2001. Cultural and genetic evolution in mountain white-crowned sparrows: song dialects are associated with population structure. Evolution; international journal of organic evolution 55:2568–75.

Maguire, S. E., and R. J. Safran. 2010. Morphological and genetic predictors of parental care in the North American barn swallow Hirundo rustica erythrogaster. Journal of Avian Biology 41:74–82.

Marler, P. 1997. Three models of song learning: Evidence from behavior. Journal of Neurobiology 33:501–516.

Marler, P., and H. W. Slabbekoorn. 2004. Nature's Music: The Science of Birdsong (Vol. 1). Elsevier, Boston.

Marshall, D. C., and J. R. Cooley. 2000. Reproductive character displacement and speciation in periodical cicadas, with description of a new species, 13-year magicicada neotredecim. Evolution 54:1313–1325.

Marshall, D. C., K. Slon, J. R. Cooley, K. B. R. Hill, and C. Simon. 2008. Steady Plio-Pleistocene diversification and a 2-million-year sympatry threshold in a New Zealand cicada radiation. Molecular Phylogenetics and Evolution 48:1054–66.

Martin, M. D., and T. C. Mendelson. 2012. Signal Divergence is Correlated with Genetic Distance and not Environmental Differences in Darters (Percidae: Etheostoma). Evolutionary Biology 39:231–241.

Masta, S. E., and W. P. Maddison. 2002. Sexual selection driving diversification in jumping spiders. Proceedings of the National Academy of Sciences of the United States of America 99:4442–7.

McComb, K. 1991. Female choice for high roaring rates in red deer, Cervus elaphus. Animal Behaviour 41:79–88.

McDonald, D. B., and W. K. Potts. 1994. Cooperative display and relatedness among males in a lek-mating bird. Science 266:1030–1032.

Mcgregor, P. K., J. R. Krebs, and C. M. Perrins. 1981. Song Repertoires and Lifetime Reproductive Success in the Great Tit (Parus major). The American Naturalist 118:149–159.

McNett, G. D., and R. B. Cocroft. 2008. Host shifts favor vibrational signal divergence in Enchenopa binotata treehoppers. Behavioral Ecology 19:650–656.

Mendelson, T. C., and K. L. Shaw. 2002. Genetic and behavioral components of the cryptic species boundary between Laupala cerasina and L. kohalensis (Orthoptera: Gryllidae). Genetica 116:301–10.

Mendelson, T. C., and K. L. Shaw. 2005. Sexual behaviour: rapid speciation in an arthropod. Nature 433:375–6.

Mendelson, T. C., and K. L. Shaw. 2006. Close-range acoustic signaling and mate choice in Hawaiian crickets (Gryllidae: Laupala). Behavioral Ecology and Sociobiology 59:770–776.

Mendelson, T. C., and K. L. Shaw. 2012. The (mis)concept of species recognition. Trends in Ecology & Evolution 27:421–427.

Mennill, D. J., L. M. Ratcliffe, and P. T. Boag. 2002. Female eavesdropping on male song contests in songbirds. Science (New York, N.Y.) 296:873.

Møller, A. 1985. Mixed reproductive strategy and mate guarding in a semi-colonial passerine, the swallow Hirundo rustica. Behavioral Ecology and Sociobiology 17:401–408.

Møller, A. 1991. Parasite load reduces song output in a passerine bird. Animal Behaviour 41:723–730.

Møller, A. P. 1988. Female choice selects for male sexual tail ornaments in the monogamous swallow. Nature 332:640–642.

Møller, A. P. 1994. Sexual Selection and the Barn Swallow. Oxford University Press.

Møller, A. P., Y. Chabi, J. J. Cuervo, F. De Lope, J. Kilpimaa, M. Kose, P. Matyjasiak, et al. 2006. An analysis of continent-wide patterns of sexual selection in a passerine bird. Evolution; international journal of organic evolution 60:856–68.

Møller, A. P., P. Y. Henry, and J. Erritzøe. 2000. The evolution of song repertoires and immune defence in birds. Proceedings of the Royal Society B: Biological Sciences 267:165–9.

Møller, A. P., N. Saino, G. Taramino, P. Galeotti, and S. Ferrario. 1998. Paternity and Multiple Signaling: Effects of a Secondary Sexual Character and Song on Paternity in the Barn Swallow. The American Naturalist 151:236–242.

Møller, A., and A. Pomiankowski. 1993. Why have birds got multiple sexual ornaments? Behavioral Ecology and Sociobiology 32:167–176.

Moreno, J., J.-G. Martínez, J. Morales, E. Lobato, S. Merino, G. Tomás, R. a. Vásquez, et al. 2010. Paternity Loss in Relation to Male Age, Territorial Behaviour and Stress in the Pied Flycatcher. Ethology 116:76–84.

Morton, E. S. 1975. Sources of Selection on Avian Sounds. The American Naturalist 109:17–34.

Moseley, D., D. Lahti, and J. Podos. 2013. Responses to song playback vary with the vocal performance of both signal senders and receivers. Proceedings of the Royal Society B: Biological Sciences 280:20131401.

Naguib, M. 2003. Reverberation of rapid and slow trills: Implications for signal adaptations to long-range communication. The Journal of the Acoustical Society of America 113:1749.

Nakagawa, S., and I. C. Cuthill. 2007. Effect size, confidence interval and statistical significance: a practical guide for biologists. Biological Reviews of the Cambridge Philosophical Society 82:591–605.

Nakagawa, S., and H. Schielzeth. 2013. A general and simple method for obtaining R^2 from generalized linear mixed-effects models. Methods in Ecology and Evolution 4:133–142.

Neuman, C. R., R. J. Safran, and I. J. Lovette. 2007. Male tail streamer length does not predict apparent or genetic reproductive success in North American barn swallows Hirundo rustica erythrogaster. Journal of Avian Biology 38:28–36.

Nevo, E. 1973. Adaptive variation in size of cricket frogs. Ecology 54:1271–1281.

Noh, S., and C. S. Henry. 2010. Sexually monomorphic mating preferences contribute to premating isolation based on song in European green lacewings. Evolution 64:261–70.

Noor, M. A. F., and J. L. Feder. 2006. Speciation genetics: evolving approaches. Nature Reviews. Genetics 7:851–61.

Nosil, P., and S. M. Flaxman. 2011. Conditions for mutation-order speciation. Proceedings of the Royal Society B: Biological Sciences 278:399–407.

Nowicki, S., and W. Searcy. 2005. Song and mate choice in birds: how the development of behavior helps us understand function. The Auk 122:1–14.

Nowicki, S., and W. A. Searcy. 2004. Song function and the evolution of female preferences: why birds sing, why brains matter. Annals of the New York Academy of Sciences 1016:704–23.

Oberweger, K., and F. Goller. 2001. The metabolic cost of birdsong production. The Journal of Experimental Biology 204:3379–88.

Olofsson, H., A. M. Frame, and M. R. Servedio. 2011. Can Reinforcement Occur With a Learned Trait? Evolution 65:1992–2003.

Ord, T. J. 2012. Receiver perception predicts species divergence in long-range communication. Animal Behaviour 83:3–10.

Orteiza, N., J. E. Linder, and W. R. Rice. 2005. Sexy sons from re-mating do not recoup the direct costs of harmful male interactions in the Drosophila melanogaster laboratory model system. Journal of evolutionary biology 18:1315–23.

Otter, K. a., I. R. K. Stewart, P. K. McGregor, A. M. R. Terry, T. Dabelsteen, and T. Burke. 2001. Extra-pair paternity among Great Tits Parus major following manipulation of male signals. Journal of Avian Biology 32:338–344.

Otter, K., P. K. McGregor, A. M. R. Terry, F. R. L. Burford, T. M. Peake, and T. Dabelsteen. 1999. Do female great tits (Parus major) assess males by eavesdropping? A field study using interactive song playback. Proceedings of the Royal Society B: Biological Sciences 266:1305–1309.

Päckert, M., J. Martens, S. Eck, A. A. Nazarenko, O. P. Valchuk, B. Petri, and M. Veith. 2005. The great tit (Parus major) – a misclassified ring species. Biological Journal of the Linnean Society 86:153–174.

Page, R., and M. Ryan. 2008. The effect of signal complexity on localization performance in bats that localize frog calls. Animal Behaviour 76:761–769.

Panhuis, T. M., R. Butlin, M. Zuk, and T. Tregenza. 2001. Sexual selection and speciation. Trends in ecology & evolution 16:364–371.

Parker, K. A., M. E. Hauber, and D. H. Brunton. 2010. Contemporary cultural evolution of a conspecific recognition signal following serial translocations. Evolution; international journal of organic evolution 64:2431–41.

Parsons, Y. M., and K. L. Shaw. 2001. Species boundaries and genetic diversity among Hawaiian crickets of the genus Laupala identified using amplified fragment length polymorphism. Molecular Ecology 10:1765–72.

Partan, S., and P. Marler. 1999. Communication goes multimodal. Science 283:1272–1274.

Partan, S. R. 2004. Multisensory Animal Communication. In G. Calvert, C. Spence, & B. E. Stein, eds., The Handbook of Multisensory Processes (pp. 225–240). MIT Press.

Partan, S. R. 2013. Ten unanswered questions in multimodal communication. Behavioral Ecology and Sociobiology 67:1523–1539.

Partan, S. R., and P. Marler. 2005. Issues in the classification of multimodal communication signals. The American Naturalist 166:231–45.

Patten, M. A., J. T. Rotenberry, and M. Zuk. 2004. Habitat selection, acoustic adaptation, and the evolution of reproductive isolation. Evolution 58:2144–55.

Pennetier, C., B. Warren, K. R. Dabiré, I. J. Russell, and G. Gibson. 2010. "Singing on the wing" as a mechanism for species recognition in the malarial mosquito Anopheles gambiae. Current Biology 20:131–6.

Peters, S., W. Searcy, and P. Marler. 1980. Species song discrimination in choice experiments with territorial male swamp and song sparrows. Animal Behaviour 28:393–404.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R Core Team. 2013. nlme: Linear and Nonlinear Mixed Effects Models.

Pitcher, B. J., E. F. Briefer, E. Vannoni, and A. G. McElligott. 2014. Fallow bucks attend to vocal cues of motivation and fatigue. Behavioral Ecology 25:392–401.

Podos, J. 1997. A Performance Constraint on the Evolution of Trilled Vocalizations in a Songbird Family (Passeriformes: Emberizidae). Evolution 51:537–551.

Podos, J. 2001. Correlated evolution of morphology and vocal signal structure in Darwin's finches. Nature 409:185–188.

Podos, J. 2007. Discrimination of geographical song variants by Darwin's finches. Animal Behaviour 73:833–844.

Podos, J. 2010. Acoustic discrimination of sympatric morphs in Darwin's finches: a behavioural mechanism for assortative mating? Philosophical Transactions of the Royal Society of London. Series B, Biological sciences 365:1031–1039.

Podos, J., and P. S. Warren. 2007. The Evolution of Geographic Variation in Birdsong. Advances in the Study of Behaviour 37:403–458.

Price, J. 2013. Why is birdsong so repetitive? Signal detection and the evolution of avian singing modes. Behaviour 150:995–1013.

Price, J. J., and S. M. Lanyon. 2004. Patterns of song evolution and sexual selection in the oropendolas and caciques. Behavioral Ecology 15:485–497.

Price, J. J., S. M. Lanyon, and K. E. Omland. 2009. Losses of female song with changes from tropical to temperate breeding in the New World blackbirds. Proceedings. Biological sciences / The Royal Society 276:1971–80.

Price, T. 1998. Sexual selection and natural selection in bird speciation. Philosophical Transactions of the Royal Society B: Biological Sciences 353:251–260.

Price, T. 2008. Speciation in Birds. Roberts and Co. Greenwood Village, Colorado.

Prohl, H., R. A. Koshy, U. Mueller, S. A. Rand, M. J. Ryan, and A. S. Rand. 2006. Geographic variation of genetic and behavioral traits in northern and southern tungara frogs. Evolution 60:1669–1679.

R Core Team. 2014. R: A Language and Environment for Statistical Computing. Vienna, Austria.

Rand, A. S., and M. J. Ryan. 1981. The Adaptive Significance of a Complex Vocal Repertoire in a Neotropical Frog. Zeitschrift für Tierpsychologie 57:209–214.

Ratcliffe, L. M., and P. R. Grant. 1985. Species recognition in Darwin's finches (Geospiza, Gould). III . Male responses to playback of different song types, dialects and heterospecific songs. Animal Behaviour 33:290–307.

Reby, D., and K. McComb. 2003a. Vocal Communication and Reproduction in Deer. Advances in the Study of Behavior 33:231–264.

Reby, D., and K. McComb. 2003b. Anatomical constraints generate honesty: acoustic cues to age and weight in the roars of red deer stags. Animal Behaviour 65:519–530.

Reby, D., K. McComb, B. Cargnelutti, C. Darwin, W. T. Fitch, and T. Clutton-Brock. 2005. Red deer stags use formants as assessment cues during intrasexual agonistic interactions. Proceedings. Biological sciences / The Royal Society 272:941–7.

Reid, J. M., P. Arcese, A. L. E. V Cassidy, S. M. Hiebert, J. N. M. Smith, P. K. Stoddard, A. B. Marr, et al. 2005a. Fitness correlates of song repertoire size in free-living song sparrows (Melospiza melodia). The American Naturalist 165:299–310.

Reid, J. M., P. Arcese, A. L. E. V Cassidy, A. B. Marr, J. N. M. Smith, and L. F. Keller. 2005b. Hamilton and Zuk meet heterozygosity? Song repertoire size indicates inbreeding and immunity in song sparrows (Melospiza melodia). Proceedings of the Royal Society B: Biological Sciences 272:481–7.

Reinhold, K. 2011. Variation in acoustic signalling traits exhibits footprints of sexual selection. Evolution 65:738–45.

Richards, S. A., M. J. Whittingham, and P. A. Stephens. 2011. Model selection and model averaging in behavioural ecology: the utility of the IT-AIC framework. Behavioral Ecology and Sociobiology 65:77–89.

Ritchie, M. G. 2007. Sexual Selection and Speciation. Annual Review of Ecology, Evolution, and Systematics 38:79–102.

Ritchie, M. G., and C. P. Kyriacou. 1994. Genetic variability of courtship song in a population of Drosophila melanogaster. Animal Behaviour 48:425–434.

Ritchie, M., E. Halsey, and J. Gleason. 1999. Drosophila song as a species-specific mating signal and the behavioural importance of Kyriacou & Hall cycles in D. melanogaster song. Animal Behaviour 58:649–657.

Rodríguez, R. L., K. Ramaswamy, and R. B. Cocroft. 2006. Evidence that female preferences have shaped male signal evolution in a clade of specialized plant-feeding insects. Proceedings. Biological sciences / The Royal Society 273:2585–93.

Rodriguez, R. L., L. E. Sullivan, and R. B. Cocroft. 2004. Vibrational Communication and Reproductive Isolation in the Enchenopa binotata Species Complex of Treehoppers (Hemiptera: Membracidae). Evolution 58:571–578.

Rodríguez, R. L., L. M. Sullivan, R. L. Snyder, and R. B. Cocroft. 2008. Host shifts and the beginning of signal divergence. Evolution 62:12–20.

Rodríguez-Muñoz, R., A. Bretman, J. Slate, C. a Walling, and T. Tregenza. 2010. Natural and sexual selection in a wild insect population. Science 328:1269–72.

Romer, H. 1993. Environmental and Biological Constraints for the Evolution of Long-Range Signalling and Hearing in Acoustic Insects. Philosophical Transactions of the Royal Society B: Biological Sciences 340:179–185.

Rowe, C. 1999. Receiver psychology and the evolution of multicomponent signals. Animal Behaviour 58:921–931.

Ruegg, K., H. Slabbekoorn, S. Clegg, and T. B. Smith. 2006. Divergence in mating signals correlates with ecological variation in the migratory songbird, Swainson's thrush (Catharus ustulatus). Molecular ecology 15:3147–56.

Ryan, M. J. 1980. Female Mate Choice in a Neotropical Frog. Science 209:523–525.

Ryan, M. J. 1983. Sexual Selection and Communication in a Neotropical Frog , Physalaemus pustulosus. Evolution 37:261–272.

Ryan, M. J., X. E. Bernal, and a S. Rand. 2007. Patterns of mating call preferences in túngara frogs, Physalaemus pustulosus. Journal of Evolutionary Biology 20:2235–47.

Ryan, M. J., and E. A. Brenowitz. 1985. The Role of Body Size, Phylogeny, and Ambient Noise in the Evolution of Bird Song. The American Naturalist 126:87–100.

Ryan, M. J., R. B. Cocroft, and W. Wilczynski. 1990a. The Role of Environmental Selection in Intraspecific Divergence of Mate Recognition Signals in the Cricket Frog, Acris crepitans. Evolution 44:1869–1872.

Ryan, M. J., J. H. Fox, W. Wilczynski, and A. S. Rand. 1990b. Sexual selection for sensory exploitation in the frog Physalaemus pustulosus. Nature 343:66–67.

Ryan, M. J., S. A. Perrill, and W. Wilczynski. 1992. Auditory tuning and call frequency predict population-based mating preferences in the cricket frog, Acris crepitans. The American Naturalist 139:1370–1383.

Ryan, M. J., and A. S. Rand. 1993a. Sexual selection and signal evolution: the ghost of biases past. Evolution 47:187–195.

Ryan, M. J., and A. S. Rand. 1993b. Phylogenetic patterns of behavioral mate recognition systems in the Physalaemus pustulosus species group (Anura: Leptodactylidae): the role of ancestral and derived characters and sensory exploitation. Evolutionary Patterns and Processes. Linnean Society Symposium Series (Vol. 14, pp. 251–267).

Ryan, M. J., and A. S. Rand. 1993c. Species Recognition and Sexual Selection as a Unitary Problem in Animal Communication. Evolution 47:647–657.

Ryan, M. J., and A. S. Rand. 1999. Phylogenetic influence on mating call preferences in female túngara frogs, Physalaemus pustulosus. Animal Behaviour 57:945–956.

Ryan, M. J., A. S. Rand, and L. A. Weigt. 1996. Allozyme and Advertisement Call Variation in the Tungara Frog, Physalaemus pustulosus. Evolution 50:2435–2453.

Ryan, M., and A. Rand. 1998. Evoked vocal response in male túngara frogs: pre-existing biases in male responses? Animal behaviour 56:1509–1516.

Safran, R., J. Adelman, K. McGraw, and M. Hau. 2008. Sexual signal exaggeration affects physiological state in male barn swallows. Current Biology 18:461–462.

Safran, R., S. Flaxman, M. Kopp, D. E. Irwin, D. Briggs, M. R. Evans, W. C. Funk, et al. 2012. A robust new metric of phenotypic distance to estimate and compare multiple trait differences among populations. Current Zoology 58:426–439.

Safran, R. J., and K. J. McGraw. 2004. Plumage coloration, not length or symmetry of tail-streamers, is a sexually selected trait in North American barn swallows. Behavioral Ecology 15:455–461.

Safran, R. J., K. J. McGraw, M. R. Wilkins, J. K. Hubbard, and J. Marling. 2010. Positive carotenoid balance correlates with greater reproductive performance in a wild bird. PloS ONE 5:e9420.

Safran, R., C. R. Neuman, K. J. McGraw, and I. J. Lovette. 2005. Dynamic paternity allocation as a function of male plumage color in barn swallows. Science 309:2210–2.

Saino, N., A. M. Bolzern, A. P. Møller, and G. H. Orians. 1997a. Immunocompetence, ornamentation, and viability of male barn swallows (Hirundo rustica). Proceedings of the National Academy of Sciences 94:549–52.

Saino, N., P. Galeotti, R. Sacchi, and A. P. Møller. 1997b. Song and immunological condition in male barn swallows (Hirundo rustica). Behavioral Ecology 8:364–371.

Saino, N., C. R. Primmer, H. Ellegren, and A. P. Møller. 1997c. An Experimental Study of Paternity and Tail Ornamentation in the Barn Swallow (Hirundo rustica). Evolution 51:562.

Saino, N., M. Romano, R. Sacchi, P. Ninni, P. Galeotti, and A. P. Møller. 2003. Do male barn swallows (Hirundo rustica) experience a trade-off between the expression of multiple sexual signals? Behavioral Ecology and Sociobiology 54:465–471.

Sauer, J., and B. Hausdorf. 2009. Sexual selection is involved in speciation in a land snail radiation on crete. Evolution 63:2535–46.

Schaefer, H. M. 2006. The role of chromatic and achromatic signals for fruit detection by birds. Behavioral Ecology 17:784–789.

Schmidt, R., H. P. Kunc, V. Amrhein, and M. Naguib. 2008. Aggressive responses to broadband trills are related to subsequent pairing success in nightingales. Behavioral Ecology 19:635–641.

Schmidt, R. S. 1965. Larynx Control and Call Production in Frogs. Copeia 1965:143.

Searcy, W. a. 1984. Song repertoire size and female preferences in song sparrows. Behavioral Ecology and Sociobiology 14:281–286.

Searcy, W. A. 1992. Song Repertoire and Mate Choice in Birds. Integrative and Comparative Biology 32:71–80.

Searcy, W. A., and M. D. Beecher. 2009. Song as an aggressive signal in songbirds. Animal Behaviour 78:1281–1292.

Searcy, W. A., S. Nowicki, M. Hughes, and S. Peters. 2002. Geographic song discrimination in relation to dispersal distances in song sparrows. The American Naturalist 159:221–30.

Seddon, N. 2005. Ecological Adaptation and Species Recognition Drives Vocal Evolution in Neotropical Suboscine Birds. Evolution 59:200–215.

Seddon, N., C. A. Botero, J. A. Tobias, P. O. Dunn, H. E. A. Macgregor, D. R. Rubenstein, J. A. C. Uy, et al. 2013. Sexual selection accelerates signal evolution during speciation in birds. Proceedings of the Royal Society B: Biological Sciences 280.

Seddon, N., R. M. Merrill, and J. A. Tobias. 2008. Sexually selected traits predict patterns of species richness in a diverse clade of suboscine birds. The American Naturalist 171:620–31.

Seddon, N., and J. Tobias. 2007. Song divergence at the edge of Amazonia: an empirical test of the peripatric speciation model. Biological Journal of the Linnean Society 90:173–188.

Seddon, N., and J. a Tobias. 2010. Character displacement from the receiver's perspective: species and mate recognition despite convergent signals in suboscine birds. Proceedings of the Royal Society B: Biological Sciences 277:2475–83.

Seehausen, O., Y. Terai, I. S. Magalhaes, K. L. Carleton, H. D. J. Mrosso, R. Miyagi, I. van der Sluijs, et al. 2008. Speciation through sensory drive in cichlid fish. Nature 455:620–6.

Servedio, M. R., G. S. Van Doorn, M. Kopp, A. M. Frame, and P. Nosil. 2011. Magic traits in speciation: "magic" but not rare? Trends in Ecology & Evolution 26:389–397.

Shaw, K. L. 1996. Polygenic Inheritance of a Behavioral Phenotype : Interspecific Genetics of Song in the Hawaiian Cricket Genus Laupala. Evolution 50:256–266.

Shaw, K. L., C. K. Ellison, K. P. Oh, and C. Wiley. 2011. Pleiotropy, "sexy" traits, and speciation. Behavioral Ecology 22:1154–5.

Shaw, K. L., and D. P. Herlihy. 2000a. Acoustic preference functions and song variability in the Hawaiian cricket Laupala cerasina. Proceedings. Biological sciences / The Royal Society 267:577–84.

Shaw, K. L., and D. P. Herlihy. 2000b. Acoustic preference functions and song variability in the Hawaiian cricket Laupala cerasina. Proceedings of the Royal Society B: Biological Sciences 267:577–84.

Shaw, K. L., and S. C. Lesnick. 2009. Genomic linkage of male song and female acoustic preference QTL underlying a rapid species radiation. Proceedings of the National Academy of Sciences of the United States of America 106:9737–42.

Smith, C. L., and C. S. Evans. 2013. A new heuristic for capturing the complexity of multimodal signals. Behavioral Ecology and Sociobiology 67:1389–1398.

Smith, H. G., and R. Montgomerie. 1992. Male Incubation in Barn Swallows: The Influence of Nest Temperature and Sexual Selection. The Condor 94:750–759.

Smith, H., and R. Montgomerie. 1991. Sexual selection and the tail ornaments of North American barn swallows. Behavioral Ecology and Sociobiology 28:195–201.

Smith, H., R. Montgomerie, and T. Põldman. 1991. DNA fingerprinting reveals relation between tail ornaments and cuckoldry in barn swallows, Hirundo rustica. Behavioral Ecology 2:90–98.

Smith, M., and D. Harper. 1995. Animal signals: models and terminology. Journal of Theoretical iology 177:305–311.

Snell-Rood, E. C., and A. V Badyaev. 2008. Ecological gradient of sexual selection: elevation and song elaboration in finches. Oecologia 157:545–51.

Sosa-López, J. R., and D. J. Mennill. 2014. Continent-wide patterns of divergence in acoustic and morphological traits in the House Wren species complex. The Auk 131:41–54.

Spencer, K. 2003. Song as an honest signal of developmental stress in the zebra finch (Taeniopygia guttata). Hormones and Behavior 44:132–139.

Sprau, P., R. Schmidt, T. Roth, V. Amrhein, and M. Naguib. 2010. Effects of Rapid Broadband Trills on Responses to Song Overlapping in Nightingales. Ethology 116:300–308.

Stewart, K. A., and E. A. MacDougall-Shackleton. 2008. Local song elements indicate local genotypes and predict physiological condition in song sparrows Melospiza melodia. Biology Letters 4:240–2.

Stewart, K. A., E. A. Macdougall-shackleton, and A. Elizabeth. 2009. Local song elements indicate local genotypes and predict physiological condition in song sparrows Melospiza melodia Local song elements indicate local genotypes and predict physiological condition in song sparrows Melospiza melodia 240–242.

Stoffer, B., and S. E. Walker. 2012. The use of multimodal communication in mate choice decisions by female house crickets, Acheta domesticus. Animal Behaviour 83:1131–8.

Stuart-Fox, D., and I. P. F. Owens. 2003. Species richness in agamid lizards: chance, body size, sexual selection or ecology? Journal of Evolutionary Biology 16:659–69.

Sullivan-Beckers, L., and R. B. Cocroft. 2010. The importance of female choice, male-male competition, and signal transmission as causes of selection on male mating signals. Evolution 64:3158–71.

Symonds, M. R. E., and A. Moussalli. 2010. A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. Behavioral Ecology and Sociobiology 65:13–21.

Taff, C. C., C. R. Freeman-Gallant, P. O. Dunn, and L. A. Whittingham. 2013. Spatial distribution of nests constrains the strength of sexual selection in a warbler. Journal of Evolutionary Biology 26:1392–405.

Taff, C. C., K. A. Littrell, and C. R. Freeman-Gallant. 2012. Female Song in the Common Yellowthroat. The Wilson Journal of Ornithology 124:370–374.

Talyn, B. C., and H. B. Dowse. 2004. The role of courtship song in sexual selection and species recognition by female Drosophila melanogaster. Animal Behaviour 68:1165–1180.

Taylor, R. C., B. A. Klein, and M. J. Ryan. 2011. Inter-signal interaction and uncertain information in anuran multimodal signals. Current Zoology 57:153–161.

Tinghitella, R. M., and M. Zuk. 2009. Asymmetric mating preferences accommodated the rapid evolutionary loss of a sexual signal. Evolution 63:2087–98.

Tobias, J. A., J. Aben, R. T. Brumfield, E. P. Derryberry, W. Halfwerk, H. Slabbekoorn, and N. Seddon. 2010. Song divergence by sensory drive in Amazonian birds. Evolution 64:2820–39.

Tobias, J. A., V. Gamarra-Toledo, D. García-Olaechea, P. C. Pulgarín, and N. Seddon. 2011. Year-round resource defence and the evolution of male and female song in suboscine birds: social armaments are mutual ornaments. Journal of Evolutionary Biology 24:2118–38.

Tobias, J. A., R. Montgomerie, and B. E. Lyon. 2012. The evolution of female ornaments and weaponry: social selection, sexual selection, and ecological competition.

Tobias, J. A., and N. Seddon. 2009. Signal design and perception in Hypocnemis antbirds: evidence for convergent evolution via social selection. Evolution 63:3168–89.

Toews, D. P. L., and D. E. Irwin. 2008. Cryptic speciation in a Holarctic passerine revealed by genetic and bioacoustic analyses. Molecular Ecology 17:2691–705.

Tsyusko, O. V., M. B. Peters, C. Hagen, T. D. Tuberville, T. a. Mousseau, A. P. Møller, and T. C. Glenn. 2007. Microsatellite markers isolated from barn swallows (Hirundo rustica). Molecular Ecology Notes 7:833–835.

Turner, A. 2010. The barn swallow. T & AD Poyser, London, UK.

Tuttle, M. D., and M. J. Ryan. 1981. Bat Predation and the Evolution of Frog Vocalizations in the Neotropics. Science 214:677–678.

Uy, J. A. C., and R. J. Safran. 2013. Variation in the temporal and spatial use of signals and its implications for multimodal communication. Behavioral Ecology and Sociobiology 67:1499–1511.

Vallet, E., I. Beme, and M. Kreutzer. 1998. Two-note syllables in canary songs elicit high levels of sexual display. Animal Behaviour 55:291–7.

Van Doorn, G. S., P. Edelaar, and F. J. Weissing. 2009. On the origin of species by natural and sexual selection. Science 326:1704–7.

Vannoni, E., and A. G. McElligott. 2008. Low frequency groans indicate larger and more dominant fallow deer (Dama dama) males. PloS ONE 3:e3113.

Venables, W. N., and B. D. Ripley. 2002. Modern Applied Statistics with S (Fourth.). Springer, New York.

Vincent, C. M., and S. M. Bertram. 2010. Crickets groom to avoid lethal parasitoids. Animal Behaviour 79:51–56.

Vitousek, M. N., R. A. Stewart, and R. J. Safran. 2013. Female plumage colour influences seasonal oxidative damage and testosterone profiles in a songbird. Biology letters 9:20130539.

Vortman, Y., a. Lotem, R. Dor, I. J. Lovette, and R. J. Safran. 2011. The sexual signals of the East-Mediterranean barn swallow: a different swallow tale. Behavioral Ecology 22:1344–1352.

Vortman, Y., A. Lotem, R. Dor, I. Lovette, and R. J. Safran. 2013. Multiple sexual signals and behavioral reproductive isolation in a diverging population. The American naturalist 182:514–23.

Wagner, W. E. 1996. Convergent song preferences between female field crickets and acoustically orienting parasitoid flies. Behavioral Ecology 7:279–285.

Wagner, W. E., and A. L. Basolo. 2007a. The relative importance of different direct benefits in the mate choices of a field cricket. Evolution 61:617–22.

Wagner, W. E., and A. L. Basolo. 2007b. Host preferences in a phonotactic parasitoid of field crickets: the relative importance of host song characters. Ecological Entomology 32:478–484.

Wagner, W. E., and M. G. Reiser. 2000. The importance of calling song and courtship song in female mate choice in the variable field cricket. Animal Behaviour 59:1219–1226.

Wagner, William E., J. 1989. Social correlates of variation in male calling behavior in Blanchard's cricket frog, Acris crepitans blanchardi. Ethology 82:27–45.

Wagner, William E., J., A.-M. Murray, and W. H. Cade. 1995. Phenotypic variation in the mating preferences of female field crickets, Gryllus integer. Animal Behaviour 49:1269–1281.

Ward, S., H. M. Lampe, and P. J. B. Slater. 2004. Singing is not energetically demanding for pied flycatchers, Ficedula hypoleuca. Behavioral Ecology 15:477–484.

Weir, J. T., and D. Wheatcroft. 2011. A latitudinal gradient in rates of evolution of avian syllable diversity and song length. Proceedings of the Royal Society B: Biological Sciences 278:1713–20.

West-Eberhard, M. J. 1983. Sexual selection, social competition, and speciation. Quarterly Review of Biology 58:155–183.

Westneat, M. W., J. H. Long, W. Hoese, and S. Nowicki. 1993. Kinematics of birdsong: functional correlation of cranial movements and acoustic features in sparrows. The Journal of Experimental Biology 182:147–71.

Whittingham, M. J., P. A. Stephens, R. B. Bradbury, and R. P. Freckleton. 2006. Why do we still use stepwise modelling in ecology and behaviour? The Journal of Animal Ecology 75:1182–9.

Wilczynski, W., A. S. Rand, and M. J. Ryan. 1995. The processing of spectral cues by the call analysis system of the túngara frog , Physalaemus pustulosus. Animal Behaviour 49:911–929.

Wilkins, M. R., N. Seddon, and R. J. Safran. 2013. Evolutionary divergence in acoustic signals: causes and consequences. Trends in Ecology & Evolution 28:156–166.

Wilson, A. J., M. Dean, and J. P. Higham. 2013. A game theoretic approach to multimodal communication. Behavioral Ecology and Sociobiology 67:1399–1415.

Wilson, D. R., P.-P. Bitton, J. Podos, and D. J. Mennill. 2014. Uneven Sampling and the Analysis of Vocal Performance Constraints. The American Naturalist 183:214–228.

Wong, B. B. M., and U. Candolin. 2005. How is female mate choice affected by male competition? Biological Reviews of the Cambridge Philosophical Society 80:559–71.

Wood, T. K., and S. I. Guttman. 1983. Enchenopa binotata Complex: Sympatric Speciation? Science (New York, N.Y.) 220:310–2.

Woodgate, J. L., M. M. Mariette, A. T. D. Bennett, S. C. Griffith, and K. L. Buchanan. 2012. Male song structure predicts reproductive success in a wild zebra finch population. Animal Behaviour 83:773–781.

Zann, R. 1993. Variation in Song Structure within and among Populations of Australian Zebra Finches. The Auk 110:716–726.

Zink, R. M., A. Pavlova, S. Rohwer, and S. V Drovetski. 2006. Barn swallows before barns: population histories and intercontinental colonization. Proceedings of the Royal Society B: Biological Sciences 273:1245–51.

Zuk, M., and G. R. Kolluru. 1998. Exploitation of Sexual Signals by Predators and Parasitoids. The Quarterly Review of Biology 73:415–438.

8. APPENDIX

8A2.1 Glossary of terms

Acoustic Adaptation Hypothesis: nested within the sensory drive framework, predicts that habitat structural differences influence signal evolution through effects on signal transmission.
Acoustic window: the acoustic parameter space which is available for signal evolution in a given taxon in a given habitat; multidimensional axes of this window, including amplitude, pitch, and temporal signal characteristics, might show different patterns of constraint on the transmission or audibility of different acoustic features within a particular environment.
Cultural drift: changes in the composition of culturally acquired and transmitted signals in a population which are due to random differences in which variants are learned and reproduced.
Dobzhansky-Muller incompatibilities: deleterious interactions resulting when alleles which have diverged in independent lines are brought together in a hybrid genome.
Ecologically-based assortative mating: individuals mate by ecotypes; acoustic signals used in mating are associated with ecological benefits; this contrasts with preference-based mating, which does not require correlations between mating signals and traits related to ecological adaptations.
Genetic drift: changes in gene frequencies in a population due to random differences in survival and reproduction of individuals or sampling error of alleles in small population sizes.
Magic trait: a trait involved in divergent ecological adaptation which has a pleiotropic effect on reproductive isolation via assortative mating.
Mutation-order speciation: process by which different, incompatible alleles fix among populations adapting to similar environments, resulting in reproductive isolation.
Ecological selection: differential survival and reproduction as a result of genetic adaptation to a particular environment.
Preference-based mating: mate-selection is based on sexually-selected acoustic traits which are not necessarily linked to ecological adaptation.
Reinforcement: the strengthening of pre-mating barriers between incipient species in response to reduced fitness of interspecific matings.
Reproductive character displacement: pronounced differences in mating signals found in sympatry with another species, compared to the signal found in allopatry; differences can arise from increased divergence or convergence.
Sensory drive: predicts that signals, sensory systems, and microhabitat choice coevolve as a function of habitat structure, ambient noise profiles, presence of predators and parasitoids, and other sensory and physiological considerations.
Sexual selection: differential reproductive success resulting from competition for mates and fertilizations.
Species recognition: process through which individuals modulate behavior based on cues that differ between populations.

Table 8A2.2

Supplementary Table: Summary of common study organisms for acoustics and speciation (legend for abbreviations in last row)

									CONSTRAINT	ECOLOGICAL SELECTION	SEXUAL SELECTION				CONSTRAINT, ES, & SS?	DRIFT		
Organism	Substrate	Learned?	Studied in Sympatry, allopatry, Mix?	Signal differs among pops?	Signal varies w/in pops?	Discriminate among spp?	Discriminate b/w subspp (divergent pops)?	heritability known?	SD Important?	Ecological trait & signal variation correlated?	Signal var predicts pref /RS in wild?	Signal var predicts pref/ RS in lab?	Signal var correlated w/ benefits? (parental care, fecundity, etc)	Used in intrasex inxns?	Expression cost?	Drift important?	Most divergent acoustic trait	PDD
túngara frog *Engystomops pustulosus* (formerly *Physalaemus pustulosus*)	Air	No	Allopatry (Ryan et al. 2007)	Y (Ryan et al. 1996)	Y (inter-call interval, initial F, (Bosch et al. 2000) & number of chucks (Rand and Ryan 1981))	Y, (Ryan and Rand 1993b), but not all pairs (Ryan and Rand 1993c)	Y (Prohl et al. 2006)	Unk	Signal transmission properties not important (Kime et al. 2000); see expression cost for predator effects	Size - correlated with F of chuck & whine in some populations (Bosch et al. 2000); relation to ecological performance unk	Unk (although larger males have higher RS in wild, and have lower F calls)	-dominant F, +call rate(Bosch et al. 2000); Differences in F (Wilczynski et al. 1995)	Y, negative correlation b/w size and chuck(Ryan 1980)/ whine F (Bosch et al. 2000); +size= +fertility (< size diff) (Ryan 1983)	Y (Ryan and Rand 1998)	Calls used by bats to locate prey (Page and Ryan 2008); +complex call preferred by bats (Tuttle and Ryan 1981)	Not very important (Prohl et al. 2006)	F modulation (shape), call duration; also max F (Ryan and Rand 1999)	SS for sensory exploitation (Ryan et al. 1990 b)

Peters' dwarf frog *Engystomops petersi* (formerly *Physalaemus petersi*)	Air	No	Allopatry (Guerra and Ron 2008a)	Y (complexity of call) (Funk et al. 2009)	Y (Guerra and Ron 2008b)	Y (Ryan and Rand 1993b)	Y (Guerra and Ron 2008b)	Unk	Unk	Not directly tested; not supported by current evidence (Funk et al. 2009)	Unk	Y (Boul et al. 2007)	Likely similar to pustulosus	Unk	Likely similar to pustulosus	No (Boul et al. 2007)	F & call duration (Funk et al. 2008)	SS for call complexity (Boul et al. 2007)
Cricket frog *Acris blanchardi* (formerly *A. crepitans*) (Gamble et al. 2008)	Air	No	Mix (Ryan et al. 1990a)	Y (Ryan et al. 1990a)	Y (Ryan et al. 1990a)	Y (Capranica and Frishkopf 1973)	Y (Capranica and Frishkopf 1973)	Unk	Y (Ryan et al. 1990a)	Dominant F correlates with size (Wagner, William E. 1989), which relates to adaptation (Nevo 1973)	Unk	Y, + selection for lower F (Ryan et al. 1992)	Y, Dominant F correlates with size (Wagner, William E. 1989)	Y (Wagner, William E. 1989)	Unk	Unk	Dominant F, call duration & call rate (Ryan et al. 1990a)	SS and SD (Ryan et al. 1990a)
Variable field cricket *Gryllus lineaticeps*	Air	No	NA	Unk	Y, chirp rate and chirp duration (Wagner and Basolo 2007a)	Unk	Unk	Unk	Signal transmission properties unk; see expression cost for parasitoid effects	Unk	Unk	+rate, dur, amp (Wagner 1996) , (Wagner, William E. et al. 1995) , calling & courtship song chirp rate (Wagner and Reiser 2000)	Fecundity & fertility benefits correlated w/ chirp rate; longevity correlated w/ chirp duration (Wagner and Basolo 2007a)	Unk	Parasitoids prefer +rate, duration, amplitude (Wagner 1996; Wagner and Basolo 2007b); Energetic cost of + chirp rate & pulse duration (Hoback and Wagner, William E. 1997)	Unk	Pulse rate	Net effects of constraint (parasitoids) and SS (females) (Wagner 1996)

field crickets *Gryllus rubens & G. texensis* (formerly *G. integer*) (Cade and Otte 2000)	Air	No	Mix (Higgins and Waugaman 2004)	Y, nightly calling dur (Cade 1991); pulse rate (Cade and Tyshenko 1990; Higgins and Waugaman 2004)	Y, bout duration (Hedrick 1986), trill rate (Gray and Cade 1999); various traits (Higgins and Waugaman 2004)	Y (Cade and Tyshenko 1990; Higgins and Waugaman 2004)	Y (Cade and Tyshenko 1990)	bout length =0.72 (Hedrick 1988); pulses per trill=0.39(Gray and Cade 1999)	Signal transmission properties unk; see expression cost for parasitoid effects	Weight and calling duration correlated (Cade and Cade 1992), but relation to ecological performance unk	Unk	+bout duration (Hedrick 1986; Cade and Cade 1992)	Y, weight and calling duration correlated (Cade and Cade 1992); But courtship song does not correlate w/ diet, fat reserve, or residual mass (Gray and Eckhardt 2001)	Y, territory defense (Cade and Cade 1992)	Y, energetic costs (Hack 1998); also, parasitoid cost (Vincent and Bertram 2010)	Unk	Pulse rate (Higgins and Waugaman 2004), (Fitzpatrick and Gray 2001)	SS for pulse rate (Gray and Cade 1999; Gray and Cade 2000)
Organism	Substrate	Learned?	Studied in Sympatry, allopatry, Mix?	Signal differs among pops?	Signal varies w/in pops?	Discriminate among spp?	Discriminate b/w subspp (divergent pops)?	heritability known?	SD Important?	Ecological trait & signal variation correlated?	Signal var predicts pref /RS in wild?	Signal var predicts pref/ RS in lab?	Signal var correlated w/ benefits? (parental care, fecundity, etc)	Used in intrasex inxns?	Expression cost?	Drift important?	Most divergent acoustic trait	PDD
swordtail cricket *Laupala kohalensis*	Air	No	Mix (Mendelson and Shaw 2002; Mendelson and Shaw 2006)	Y (Parsons and Shaw 2001)	Y (Mendelson and Shaw 2002)	Y (Mendelson and Shaw 2002), for symp spp, not allop spp (Mendelson and Shaw 2006)	Y (Grace and Shaw 2012)	High (Shaw 1996)	Unlikely (Grace and Shaw 2011)	No ecological trait variation shown (Mendelson and Shaw 2005)	Unk	Y (Mendelson and Shaw 2002; Grace and Shaw 2012)	Unk	Unk, but likely	Unk, but likely	Unk	Pulse rate (Mendelson and Shaw 2002)	SS for pulse rate (Shaw and Lesnick 2009)

treehopper *Enchenopa binotata*	Plant stem	No	Sympatry (Wood and Guttman 1983; Rodriguez et al. 2004)	Y (Rodriguez et al. 2004)	Y (Rodriguez et al. 2004)	Y (host races) (Rodriguez et al. 2004)	Y (Rodriguez et al. 2004)	Range (~0-0.48 for pulse rate) (Rodríguez et al. 2008)	Some (McNett and Cocroft 2008), not direct (Sullivan-Beckers and Cocroft 2010)	No ecological traits studied, besides host choice	Y, natural enclosures (Sullivan-Beckers and Cocroft 2010)	Y (Rodríguez et al. 2006)	Unk	Y (Sullivan-Beckers and Cocroft 2010)	Possibly (signaling rate) (Cocroft et al. 2008), untested	Unk	Multiple temporal and F traits (Rodriguez et al. 2004; Rodríguez et al. 2006)	SS (Rodriguez et al. 2004)
green lacewing *Chrysoperla plorabunda*	Plant stem	No	Mix (Henry 1985a; Henry et al. 1999)	Y (Henry 1985a)	Y (Henry and Wells 2006)	Y (Henry 1985b)	Y, reduced hybridization (Henry 1985a)	High (Henry et al. 2002)	N (Henry and Wells 2004)	Unk	Unk	N, pref not shown to vary w trait; either spp recognition or not (Henry et al. 2002)	Unk	Y (Henry and Wells 2009)	Unk	Unk	Temporal structure (volley and signal dur) (Henry et al. 2002)	SS (Henry et al. 2002), but maybe M-O speciation
fruit fly *Drosophila melanogaster*	Air	No	Mix, as this is a globally distributed insect (Colegrave et al. 2000)	Y (Colegrave et al. 2000)	Y, (Ritchie and Kyriacou 1994)	Y (Ritchie et al. 1999)	Y (Colegrave et al. 2000)	Inter-pulse interval and F not heritable (Ritchie and Kyriacou 1994)	Unk	Unk	Unk	Y, more energetic pulse songs selected	Unk (Talyn and Dowse 2004), although direct fecundity costs shown for remating in lab (i.e. negative benefits) (Orteiza et al. 2005)	Y, signals differ by context (Jonsson et al. 2011)	Likely energetic costs (Talyn and Dowse 2004)	Unk	Inter-pulse interval (pulse tempo) (Colegrave et al. 2000)	Unk

song sparrow *Melospiza melodia*	Air	Yes	Mix (Searcy et al. 2002; Patten et al. 2004)	Y (Patten et al. 2004)	Y (Patten et al. 2004)	Y (Peters et al. 1980)	Discriminate against distant populations (Searcy et al. 2002), and diff subspp (Patten et al. 2004)	Unk	Y (Patten et al. 2004)	Unk	Y +repertoire size = +fitness) (Reid et al. 2005a)	Rep pref in lab, not pair date in field (Searcy 1984)	Y (+ fitness of young) (Reid et al. 2005a); (+immunity & outbreeding) (Reid et al. 2005b); local rep= -parasites, -stress, +loc origin (Stewart and MacDougall-Shackleton 2008)	Y (Kramer and Lemon 1983)	Sharp-shinned hawk predation (Zuk and Kolluru 1998)	Yes (Stewart and MacDougall-Shackleton 2008)	Trill tempo (cadence) has highest DFA loading in Table 3 (Patten et al. 2004)	Acoustic adaptation & drift (Patten et al. 2004)
great tit *Parus major*	Air	Yes	Mix (Päckert et al. 2005)	Y (Päckert et al. 2005)	Y (Mcgregor et al. 1981)	Unk	Unk	~0 (Mcgregor et al. 1981)	Y (Hunter and Krebs 1979)	Unk	Y (+rep=+kids) (Mcgregor et al. 1981)	Y, + solicitations for +rep (Baker et al. 1986); Results of vocal inxns don't affect EPP (Otter et al. 2001)	+rep=Kids > weight (Mcgregor et al. 1981)	Y (Otter et al. 1999)	Unk	Unk	F bandwidth, syllable makeup (Päckert et al. 2005)	SD (Hunter and Krebs 1979)

Organism	Substrate	Learned?	Studied in Sympatry, allopatry, Mix?	Signal differs among pops?	Signal varies w/in pops?	Discriminate among spp?	Discriminate b/w subspp (divergent pops)?	heritability known?	SD Important?	Ecological trait & signal variation correlated?	Signal var predicts pref/RS in wild?	Signal var predicts pref/ RS in lab?	Signal var correlated w/ benefits? (parental care, fecundity, etc)	Used in intrasex inxns?	Expression cost?	Drift important?	Most divergent acoustic trait	PDD
zebra finch *Taeniopygia guttata*	Air	Yes	Allopatry (Zann 1993)	Y (Zann 1993)	Y (Zann 1993)	Y (Campbell and Hauber 2009)	Y (Clayton 1990)	low (rep=0.08); many trait est. (Forstmeier et al. 2009)	Unk	Unk	Y (Woodgate et al. 2012)	Y, song rate(Houtman 1992);	Song characteristics reflect developmental stress (Spencer 2003); song rate correlates w/ condition (Houtman 1992)	Unk	Not so much (Oberweger and Goller 2001)	Unk	Not well characterized, but suggest elemental sequence & syllable makeup (Zann 1993)	Unk
pied & collared flycatchers *Ficedula hypoleuca* & *F. albicollis*	Air	Yes	Mix (Haavie et al. 2004)	Y (Haavie et al. 2004)	Y (Lampe and Espmark 2002)	Unk	Unk	Unk	Unk	Unk	Y (Song rate (Gottlander 1987))	Y, song rep & versatility (Lampe and Saetre 1995; Lampe and Espmark 2002)	Complexity correlated w/ condition, brighter plumage, & good territories	Y (Moreno et al. 2010)	Not energetically (Ward et al. 2004)	Unk	Tempo (Haavie et al. 2004)	Reinforcement? (Haavie et al. 2004)
winter wren *Troglodytes troglodytes*	Air	Yes	Mix (Toews and Irwin 2008)	Y (Toews and Irwin 2008)	Y (Toews and Irwin 2008)	(Amrhein and Erne 2006)	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Y, (Amrhein and Erne 2006; Toews and Irwin 2008)	Unk		Transition rate (tempo) (Toews and Irwin 2008)	SS (Toews and Irwin 2008)
greenish warbler *Phylloscopus trochiloides*	Air	Yes	Mix (ring) (Irwin et al. 2001b)	Y(Irwin et al. 2001b)	Y (Irwin et al. 2001b)	Y (Irwin et al. 2001a)	Y (Irwin et al. 2001b)	Unk	N (Irwin et al. 2008)	Unk	Unk	Y, Song rep (Gil and Slater 2000)	Unk	Y (Irwin et al. 2001a)	Unk	Y (Irwin et al. 2008)	Unit types & length (Irwin et al. 2008)	SS and drift (Irwin et al. 2008)
medium ground finch *Geospiza fortis*	Air	Yes	Mix (Podos 2007)	Y (Grant and Grant 1996)	Y (Grant and Grant 1996)	Y (Ratcliffe and Grant 1985)	Y (Podos 2010)	high transmission, father to son	Not very (Bowman 1979)	Y, beak size associated with seed-foraging and vocal performance	Unk	Unk	Unk	Y (Podos 2010)	Unk	Unk	F measures, trill rate, & vocal deviation (Huber	ES, but possibly SS? (Hub

								(Grant and Grant 1996)		(Huber and Podos 2006)						and Podos 2006)	er and Podos 2006)
Amazonian non-song-learning birds	Air	No	Mix (Tobias et al. 2010)	Y (Tobias et al. 2010)	Y (Seddon and Tobias 2010)	Y (e.g. (Seddon and Tobias 2010))	Y (e.g. (Seddon and Tobias 2007))	Unk	Y (Tobias et al. 2010)	N (Tobias et al. 2010)	Unk	Unk	Unk	Y	Unk	N (Tobias et al. 2010)	Pitch & temporal complexity (Tobias et al. 2010)	SD (Tobias et al. 2010)
red deer *Cervus elaphus*	Air	No	NA	Y (Reby and McComb 2003a)	Y (Reby and McComb 2003a)	Unk	Unk	Unk	Unk (Reby and McComb 2003a)	F correlated w/ size (Reby and McComb 2003b)	Lower minimum formant F =+RS (Reby and McComb 2003b)	Y (calls of larger males)(Charlton et al. 2007), +roar rate (McComb 1991)	Formant F correlates w/ size and age (Reby and McComb 2003b)	Y-lower formants=+aggression (Reby et al. 2005)	Unk	Unk	Unk	SS (Cap et al. 2008)

pops=populations, spp=species, subspp=subspecies, var=variation, pref=preference, inxns=interactions, ES=Ecological Selection, SS=Sexual Selection, SD= Sensory Drive, M-O=Mutation-Order, Unk=Unknown, RS=Reproductive Success, F=Frequency, PDD=Predicted Divergence Driver

8A3.1 Additional Methods

Color analyses

Feather samples from four ventral patches (throat, breast, belly, and vent) were taped to a standard white card background so that they overlapped as they do on the body of a bird. The color of each patch was measured using a spectrometer (USB 4000, Ocean Optics), pulsed xenon light (PX-2, Ocean Optics) and SpectraSuite software (v2.0.151). The probe was held at 90 degrees to the feather surface at a distance such that a 2.5 mm diameter of the surface was illuminated and measured. Each sample was measured three times, lifting the probe between measurements, and averaged. Each measurement was an average of 20 scans of the spectrometer. From the generated spectra, we calculated standard color descriptors: i) average brightness, ii) hue, and iii) red chroma. Average brightness was calculated as the average percent reflectance between 300 and 700 nm, hue was calculated as the wavelength that corresponds to where the slope of the curve is steepest between 550 and 700 nm, and red chroma was calculated as the proportion of light reflected in the red range (600 to 700 nm) relative to the entire range (300 to 700 nm).

Song analyses

Songs were recorded in 16-bit WAV format, with 48 kHz sampling rate using a Marantz PMD 660 digital recorder, paired with an Audiotechnica AT815B shotgun microphone (2009), a Marantz PMD 660 paired with a Sennheiser MKH 20 and Telinga parabola (2011), or a Marantz PMD 661 paired with a Sennheiser ME62/k6 microphone and Telinga parabola (2012). Males were identified by unique markers. Individual songs were extracted from recording sessions based on observations spoken into the directional or lapel microphone using Syrinx-PC (J. Burt, Seattle, WA). The number of rattle pulses was counted from spectrograms generated by Syrinx. All other song variables were extracted using the Automatic Parameter Measurement function of Avisoft SASLab Pro version 5.2 (www.avisoft.com, R. Specht). For temporal analysis of the rattle, we used all pulses, but for frequency analysis, we disregarded the first and last pulses because in our subspecies the frequency of these pulses was much lower than the main pulse train (figure 2B-D). We generated spectrograms for automatic parameter measurement in Avisoft (Fast Fourier Transformation = 512, Frame = 100%, Window = Hamming, Overlap = 93.75%), aiming for a balance of frequency and temporal resolution (43 Hz and 1.45 ms, respectively).

Additionally, we developed a syllable repertoire to represent all the syllables found in the total sample of 1149 songs. Following others (Catchpole and Slater 2003; Weir and Wheatcroft 2011), we defined syllables as a continuous trace on the spectrogram, except where multiple individuals produced a consistent discontinuous spectrographic pattern which was spaced much less (~0.01s) than a typical distance between syllables (~0.06s). Thus, simple sequences of syllables in one singer were sometimes combined to form a tight, compound syllable in another. Unlike previous barn swallow song studies (Galeotti et al. 1997; Garamszegi et al. 2005; Garamszegi et al. 2006a), we did not separate songs and analyses by 'Type A' and 'Type B' songs,

as song characteristics resembled a continuum of length and complexity, rather than a binary classification of types. The 51 syllables used for classification (figure A2) were based on spectrographic patterns repeated within and across individuals. Individual variation in performance of particular syllables may lead this population-level repertoire to overestimate the true number of syllable types. Therefore, repertoire estimates for this population are not comparable to other populations, but they do represent a large portion of the variation in syllable usage within and among males.

Paternity analyses:

The six microsatellite markers used for paternity analyses were Escu6: (Hanotte et al. 1994); Ltr6: (McDonald and Potts 1994); Pocc6: (Bensch et al. 1997); and Hir11, Hir19, and Hir20: (Tsyusko et al. 2007)). Reaction conditions for pooled Escu6, Ltr6, Hir20, and Hir11 primers consisted of a 10 ul solution with 50-100 ng DNA, 0.12 mM of each labeled forward primer, 0.12 mM of each reverse primer, 200 M each dNTP, 3.25 mM $MgCl_2$, 1x PCR Buffer, 0.15 units Taq polymerase (New England Biolabs, Massachusetts, U.S.A.), and were amplified with the following protocol: initial denaturation step of 94°C for 1 minute, followed by 10 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 45 s, with an additional 25 cycles starting at 87°C for 30 s instead of 94°C, and completed with a final extension at 72 °C for 3 min. The Pocc6 reaction was modified from the above conditions by using 1.25 mM $MgCl_2$, and modified for the Hir19 reaction with 3 mM $MgCl_2$ and 0.2 mM of each forward and reverse primer. The PCR amplification protocol for Pocc6 and Hir19 was similar to the pooled loci protocol with the exception that 60°C was used for the annealing temperature. Amplified PCR products containing the fluorescently-labeled forward primer were detected using an ABI3730 DNA analyzer (ABI, Inc.). Allele peaks were manually called using Peak Scanner v1.0 (Applied Biosystems) in order to minimize genotyping error associated with irregular PCR products. For the paternity analysis simulation in CERVUS, we left the proportion of loci typed at 0.959 (determined from our data) and the proportion of loci mistyped at the default 0.01, and ran the program for 10,000 iterations. For each male, we set the female seen brooding eggs as the known mother and considered an offspring as extra-pair when offspring-mother-father trio confidence did not reach the 95% level. We adopted this approach, rather than a threshold number of parent-offspring mismatches for paternity exclusions because confidence levels are derived from likelihood equations which take into account potential genotyping errors.

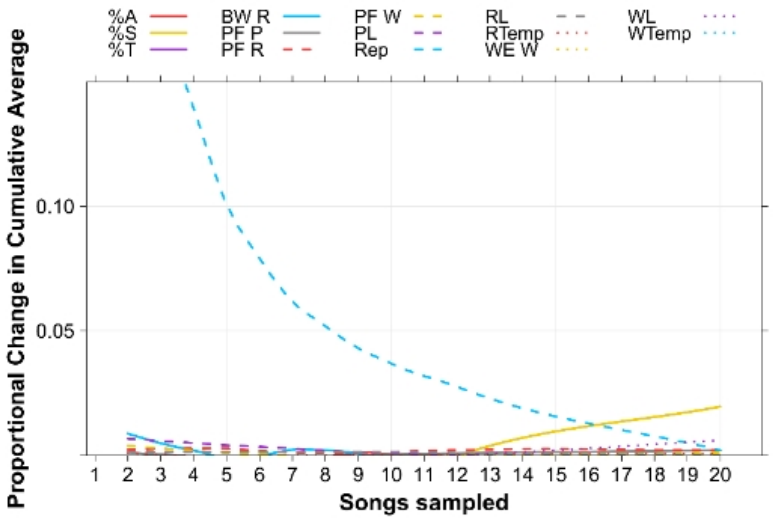

Figure 8A3.2: Rarefaction curves for song traits. Lines represent LOESS fits relating the proportionate change in each trait estimate resulting from measuring an additional song. Fits were calculated from the sample in which the most songs were recorded for each of 66 males using default settings in xyplot {lattice} in R v3.0.2.

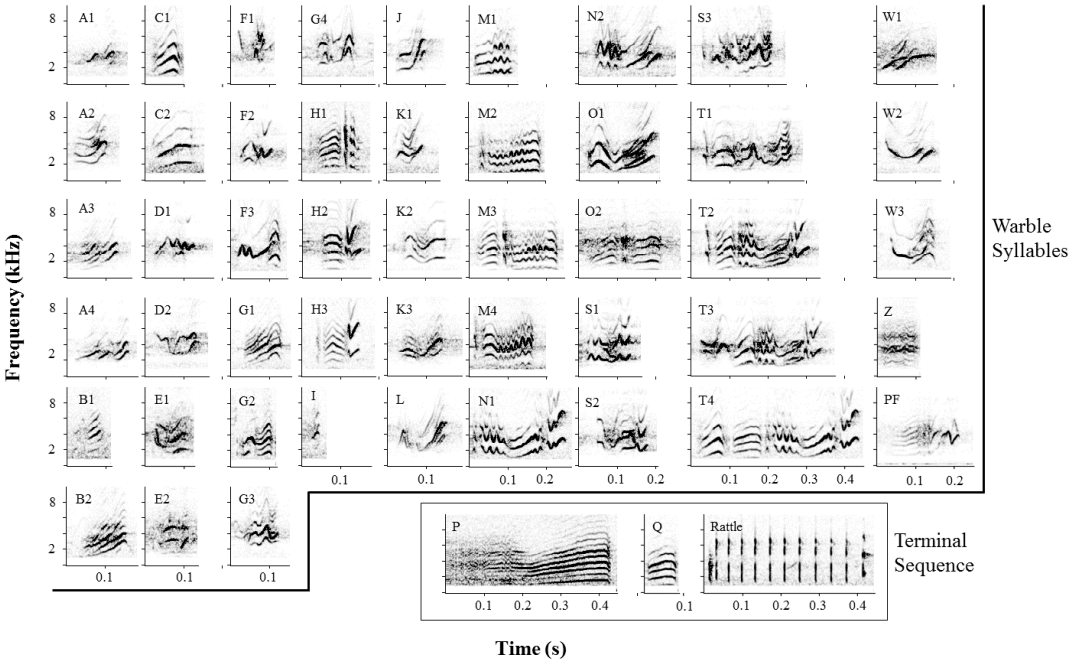

Figure 8A3.3: Examples of the 51 syllables used for classification of repertoire-related song variables in the North American barn swallow (Hirundo rustica erythrogaster).

Table 8A3.4. Rotated principal component loadings for phenotypic traits

	[Breast Paleness]	[Song Performance]	[Throat Darkness]	[Complexity/ Vent Paleness]	[Feather Length]	[Rattle Performance]	[Monotony]	[Breast Hue]	[Repertoire]
WL	-0.29	0.05	0.04	-0.01	0.19	0.05	**0.72**	0.05	0.23
PL	0.08	0.25	-0.27	0.05	-0.44	0.06	0.23	0.46	-0.05
RL	0.16	-0.17	-0.14	0.19	-0.03	**-0.79**	0.20	0.00	0.07
PF W	0.09	**0.72**	-0.07	-0.12	0.08	-0.02	0.36	-0.14	-0.13
PF P	0.18	**0.64**	-0.23	0.28	-0.32	0.25	-0.02	-0.14	-0.08
PF CR	0.19	**0.70**	-0.07	0.03	0.03	0.01	-0.48	0.06	0.21
WE W	0.29	**0.70**	0.16	0.01	-0.02	-0.08	-0.03	-0.03	0.16
BW R	0.21	**-0.64**	**0.52**	0.16	-0.02	0.03	0.15	0.16	0.07
RTmp	-0.05	-0.16	-0.04	0.09	0.04	**0.84**	0.18	0.14	-0.02
WTmp	-0.01	-0.06	0.24	-0.09	0.08	0.58	0.43	-0.06	0.25
%A	-0.02	-0.12	0.22	-0.30	0.03	0.10	**0.67**	-0.13	-0.15
%S	-0.18	**0.60**	0.22	0.32	-0.21	-0.20	-0.15	0.02	0.35
%T	-0.12	-0.02	-0.30	**0.63**	0.39	-0.14	0.05	0.06	0.19
Rep	-0.02	0.14	-0.14	-0.14	0.15	0.02	0.04	-0.12	**0.75**
RWL	0.03	-0.12	-0.19	-0.01	**0.72**	0.26	0.22	-0.05	0.01
TS	-0.11	0.06	0.06	0.15	**0.87**	-0.06	0.05	0.19	0.10
TBri	0.54	-0.04	**-0.71**	0.21	-0.07	-0.10	-0.11	0.10	0.10
THue	-0.31	0.10	**0.62**	0.20	-0.10	0.14	0.12	0.25	-0.30
TChr	-0.31	-0.11	**0.73**	-0.22	-0.05	0.07	0.11	-0.07	0.04
RBri	**0.87**	0.06	-0.08	-0.11	0.09	-0.19	-0.07	-0.03	-0.09
RHue	-0.17	-0.17	0.27	-0.05	0.01	0.10	-0.04	**0.73**	0.04
RChr	**-0.84**	0.00	0.22	0.08	-0.06	-0.05	0.15	-0.04	0.15
BBri	**0.83**	0.20	-0.16	0.20	-0.22	-0.04	0.06	-0.10	0.16
BHue	0.00	-0.11	-0.14	-0.16	0.16	0.00	-0.12	**0.75**	-0.16
BChr	**-0.86**	-0.16	0.11	-0.16	0.23	-0.01	0.08	0.12	-0.09
VBri	**0.57**	0.09	-0.07	**0.52**	0.17	-0.12	-0.13	-0.05	-0.42
VHue	-0.02	0.00	-0.10	**-0.65**	0.12	-0.05	0.26	0.35	0.15

VChr	-0.44	-0.06	0.14	**-0.68**	-0.10	0.10	0.14	0.08	0.34
Eigenvalue	4.30	3.00	2.41	2.21	2.05	2.00	1.94	1.719	1.47
Proportion Variance	0.15	0.11	0.09	0.08	0.07	0.07	0.07	0.06	0.05
Cumulative Variance	0.15	0.26	0.35	0.43	0.50	0.57	0.64	0.70	0.75

Table 8A3.5. Model-averaged estimates, standard errors, 95% confidence intervals, and importance values for fixed effects in the best models, within 2 ΔAIC_C of the top model

	2.5%	97.5%	Estimate	Std. Error	Importance
BREEDING ONSET					
[Feather Length]	-5.91	0.80	-2.55	1.71	0.369
[Breast Hue]	-0.76	6.02	2.63	1.73	0.357
[Rattle Performance]	-1.87	5.28	1.70	1.82	0.109
[Throat Darkness]	-2.44	5.54	1.55	2.04	0.094
FLEDGING					
[Breast Hue]	-0.29	-0.01	-0.15	0.07	0.796
[Repertoire]	-0.25	0.01	-0.12	0.07	0.671
PATERNITY					
[Feather Length]	0.37	1.32	0.84	0.24	1.000
[Rattle Performance]	-0.03	0.86	0.41	0.23	0.445
[Breast Paleness]	-0.91	0.03	-0.44	0.24	0.412
[Monotony]	-1.12	0.12	-0.50	0.32	0.331
[Complexity/ Vent Paleness]	0.00	0.99	0.49	0.25	0.076
DISTANCE					
[Repertoire]	0.03	0.38	0.21	0.09	1.000
[Rattle Performance]	-0.32	0.04	-0.14	0.09	0.330
[Song Performance]	-0.33	0.04	-0.15	0.10	0.318
[Breast Hue]	-0.32	0.07	-0.12	0.10	0.157